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Sporulation by *Hansenula Mrakii* on vegetable media

by

O. B. Williams and Arthur Newton
(*Department of Bacteriology, University of Texas, Austin*)

(23.9.1950)

Methods and media for inducing the formation of ascospores by yeasts have long been a subject of investigation. This has been due chiefly to the diagnostic importance of the number, mode of formation and morphology of the spore, but also to some extent because of the significance of ascospores in connection with studies in the developing field of microbial genetics. Two main lines of attack on the problem have been followed. One has been to grow the yeast on a pre-sporulation medium, transfer the vegetative cells to some non-nutrient support as plaster of Paris blocks, wood or clay blocks, filter paper, silica gel or washed agar which is kept moist with water or with some dilute solution of a test material, prepare smears at intervals and examine for the development of spores. The other procedure has been to grow the yeast directly on sporulation media, which in some instances supported luxuriant growth, and in others allowed only a limited growth. The general concensus is that no one medium or method is adequate for all types, and that there is a lack of uniformity in behavior among species, and even among strains of the same species. Phaff and Mrak (9) have recently reviewed the literature dealing with sporulation in yeasts, hence in the present instance reference need be made only to especially pertinent publications. (See also Henrici (2) and Lindegren and Lindegren (4)).

A mixed vegetable medium with an especially high spore inducing potential was described by Mrak, Phaff and Douglas (6) in 1942. This medium, which was prepared from carrots, beets, cucumbers and potatoes, came nearer being a universal sporulation medium than any previously described. However, Lindegren and Lindegren (4) found it to be ineffective with some types, and following studies to develop a more nearly optimal medium they described one containing extracts of both beet leaves and beet roots, apricot juice, grape juice, dried yeast and glycerin, and neutralized with calcium carbonate. The medium may be used either as a presporulation nutrient or directly as a sporulation medium. They did not include carrot in any of their experiments, stating that in earlier work this vegetable, which has long been used as a sporulation medium, had not been found useful.

Wickerham, Flickinger and Burton (13) described a medium, which they said was first described to them by Henrici in 1943, prepared with V—8, a commercial mixture of juices of tomato, celery, carrot, parsley, lettuce, beet, spinach and water cress, and containing salt and "seasoning." The medium has yeast added in the proportion of one half of a small yeast cake (or 10 grams of yeast) to each liter of medium. Because of ease of preparation and high spore productivity the V — 8 medium has been extensively used. Most sporulating yeasts will form spores fairly readily on the medium (Etchells (1)).

Little is known about the causal relationship to sporulation of the various components of the medium of MRAK *et al* or of the V — 8 medium. The present report gives the results of an effort to evaluate the various constituents of these media as spore inducing nutrients.

Assay organism

In a study of the comparative spore inducing potential of different nutrients the choice of an assay organism is of importance. The test organism should show on the reference or control medium a substantial percentage of the cells as asci, preferably between 35 % and 65 %, in order that significant increases or decreases in sporulation may be readily detected. It is desirable that sporulation proceed to a reproducible maximum fairly rapidly, and it is helpful in ready recognition if the organism has distinctive ascospores.

Tests on the organisms shown in the tabulation gave the percentage of asci indicated after 4 days incubation on tomato juice agar.

Saccharomyces cerevisiae	1
Saccharomyces cerevisiae var. *ellipsoideus*	1
Pichia fermentans var. *rugosa*	15
Zygosaccharomyces globiformis	25
Hansenula mrakii	32

The requirements listed were fairly well met by *Hansenula mrakii*, consequently this organism was the one selected for study.

This organism has not been officially described but a description is now in process of preparation by WICKERHAM (11) and will appear in due course in a Technical Bulletin of the U.S. Department of Agriculture. The organism has been referred to by WICKERHAM and BURTON (12) and was one of the species discussed by WICKERHAM, GILL and BURTON (14) at the 1950 meeting of the Society of American Bacteriologists.

The strain used was obtained from Dr. E. M. MRAK, of the University of California, and was maintained on nutrient agar.

Materials and methods

Vegetable base media were prepared by grinding washed unpeeled vegetables in a Waring blendor, mixing with an equal volume of distilled water, adjusting to the desired pH with a glass electrode potentiometer, adding 2 % agar and sterilizing at 15 pounds steam pressure for 15 minutes. V — 8 and tomato juice were diluted with an equal volume of distilled water and handled in a similar manner.

Petri dishes were poured and inoculated by streaking to give isolated colonies. Incubation was at 30C. Ratio counts of sporulating cells were made on smears prepared from the surface of colonies and stained by the method used by WYNNE (15) for bacterial spores. MRAK (5) has noticed for *H. mrakii* a tendency to shift from conjugation preceding sporulation to sporulation without conjugation. However, in the work here reported no tendency for sporulation without conjugation was noted.

At least 300 cells were counted on each smear from fields selected at random. At first counts were made at daily intervals but later when experience showed that maximum sporulation had occurred by the fourth day the counts were made at this interval only. The results reported are the average of percentage of asci from three determinations in close agreement.

Results

One of the conditions early recognized as of importance for ascospore formation was a free access to atmospheric oxygen, and apparently all later observations are in accord on this point. It would appear that the difference in aeration between the surface and the depths of a colony is significant since LINDEGREN and HAMILTON (3) found spores only in the surface layer of prepared cross sections of colonies of *Saccharomyces cerevisiae*. One of the preliminary experiments done as a part of the present work was an examination of smears from the surface and from the bottom levels of colonies grown on tomato juice medium for 4 days. The surface layer showed 24 % of the cells as asci, the bottom layer only 16 %. In all subsequent experiments care was used to obtain the cells for examination from the surface layer of the colony, and since results have been readily reproducible in replicate experiments it is believed that there are no errors in the results due to faulty sampling.

Other preliminary experiments included a test of the significance of the pH of the medium and of yeast extract as a component of the medium. The medium of MRAK *et al*, and V — 8 medium with 0.1 % yeast extract substituted for yeast cake, were prepared with pH values of 4.3 and of 6.8. The 4 day ascus count on the medium of MRAK *et al* was 11 and 10, and on the V — 8 medium 40 and 36 at these two pH values. It was clear that pH did not exercise a significant effect on sporulation by *H. mrakii*, and in all later experiments the media were adjusted to pH 6.8.

The importance of yeast extract as a component of the medium was tested with several media, each with 0.1 % yeast extract and with no yeast extract. The 4 day ascus count on each of the media tested was as follows:

V — 8	+	Carrot	+	Tomato	+	Spinach	+	Celery	+	Lettuce	+
Plain	y e	Plain	y e	Plain	y e	Plain	y e	Plain	y e	Plain	y e
42	40	16	12	32	32	14	13	34	34	7	8

From these data it was concluded that yeast extract in the amount used was not significant for sporulation by *H. mrakii*, and consequently yeast extract was not added to the media used in later experiments. On the basis of observations by a number of workers it might be expected that a greater amount of yeast extract would be stimulatory to sporulation, but in the interest of keeping the media as simple as possible no further tests were made. The data show very clearly that striking differences in spore productivity may be encountered among different vegetable base media.

The next experiment was designed to establish the spore productivity of the vegetable components of the MRAK *et al* and the V — 8 media. The 4 day ascus counts which were obtained are as follows:

Celery	34
Commercial tomato juice—salted	32
Commercial tomato juice—no added salt	22
Fresh tomato	21
Carrot	16
Spinach	14
Cucumber	12
Water Cress	12
Beet (root)	10
Parsley	10
Potato	9
Lettuce	7

Tables of the proximate composition of the vegetables tested were consulted to see if there were either qualitative or quantitative differences which would suggest an explanation of the results observed. However, no explanation based on this idea seemed probable. The results of tests made to determine whether the difference in count on the two lots of commercial tomato juice might be explainable on the basis of the amount of salt present did not support such an idea. Salt at a level of 2 % was inhibitory to sporulation; lesser amounts were only slightly or not at all stimulatory.

The differences in spore productivity among the various vegetable media made it seem worthwhile to test combinations to see whether the productivity of one could be altered by the addition of another. The mixtures were in equal proportions except as noted. The combinations tested and the counts recorded for each were as follows:

Carrot and celery	24
Carrot and spinach	23
Carrot and potato	18
Carrot and tomato	14
Celery and spinach	18
Celery and potato	10
Cucumber and tomato	10
Beet and tomato	15
25 % beet — 75 % tomato	14
50 % beet — 50 % tomato	14
75 % beet — 25 % tomato	15

It will be noted that in the instance of the cucumber-tomato mixture the count is essentially the same as that obtained with cucumber alone-the less productive member of the combination. The beet-tomato and carrot-celery combinations gave counts intermediate between the counts for the components alone, while the carrot-potato combination gave a count essentially the same as that of the more productive member of the pair. Such data scarcely permit of a generalization regarding the effects of one vegetable on the productivity of another, but rather speak for the necessity of testing each combination of nutrients.

The fact that growth of essentially equal luxuriance was obtained on the various vegetable base media but with significant differences in spore yield suggested either that certain of the media carried a factor(s) stimulatory to spore formation, or others a sporulation inhibitor. Several workers have noted the stimulatory effect of one or another substance (OCHMANN (8) STANTIAL (10) NICKERSON and THIMANN (7)) and a few experiments were conducted to determine whether the superiority of tomato juice might be due to the presence of a factor(s) stimulatory to spore production. Salt free juice was separated into liquid and insoluble solids fractions by centrifuging, and media prepared from each fraction. The liquid fraction supported 24 % sporulation, the solid fraction only 6 %. Further testing was done with the liquid fraction of the juice.

Control observations were made on cultures from media consisting of half strength tomato juice and gave asci counts within the range 23 to 32 %. The effect of each of the various factors tested was evaluated by incorporating treated juice, or material removed from juice, in medium in a concentration which corresponded to the amount of untreated juice in the controls.

The heat stability of the factor(s) at acid, neutral and alkaline reactions was tested by mixing two parts of juice with 1 part of 4 N hydrochloric acid or sodium hydroxide, and by adjusting a third lot to pH 6.8. These were autoclaved at 15 pounds steam pressure for 30 minutes. Each was then adjusted to pH 6.8 and incorporated in medium. Asci counts from these media gave values of 0, 13 and 23 % for the acid, neutral and alkali treated juice, respectively.

Attempts to remove the factor(s) by ether extraction at pH values of 4.4, 6.8 and 9.1 were not successful. Cultures on media containing ether extracted juice gave asci counts of 25 % while cultures from media containing an aqueous solution of the material removed by ether extraction and remaining after evaporation of the ether were negative for asci.

The active material(s) was removed by precipitation with both silver nitrate and basic lead acetate. Precipitation with silver nitrate was effected by adding a 4 % aqueous solution until no further precipitate formed, filtering and removing the excess silver with 6N hydrochloric acid. The silver chloride was filtered off and the filtrate evaporated on a steam bath to near dryness to remove the excess hydrochloric acid. The volume was restored with distilled water, and the filtrate incorporated in medium in the usual manner. Lead acetate precipitation was effected with 2 % basic lead acetate, filtering and removing the excess lead from the filtrate with hydrogen sulfide. The lead sulfide was removed by filtration and the excess hydrogen sulfide by boiling. The filtrate was restored to volume with distilled water and incorporated in medium.

Good growth was obtained on each of the media. No asci were noted on the medium containing the lead acetate precipitated juice, and less than 1 % on the silver nitrate precipitated juice.

Adsorption of juice with norite in the ratio of 1 part norite to 2 parts of soluble solids at pH 3.2, 6.8 and 9.0 showed little or no effect at pH 3.2, essentially complete removal of the factor(s) at pH 6.8 and some removal at pH 9.0. The factor(s) could not be eluted by refluxing the norite used for adsorption with either 5 % ammonia in ethanol or by a mixture of 5 % pyridine, 45 % ethanol and 50 % distilled water.

These various data are inadequate to characterize the factor(s), but do suggest that there is present in tomato juice a factor(s) stimulatory to spore production by *H. mrakii*, and that the factor(s) is probably a neutral substance(s).

Summary

A determination of the ascospore producing potential for *Hansenula mrakii* of several vegetable media, showed celery and tomato media to be much superior to all others tested, and with celery slightly better than tomato medium.

The stimulatory effect on ascospore production, at least in the instance of tomato, appears to be due to a neutral substance which is not extractable with ether, is destroyed by heating under pressure in the presence of acid, and which can be removed by norite adsorption at pH 6.8 and by precipitation with silver nitrate or basic lead acetate.

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Infezione sperimentale da *Coccidioides immitis* e steroidi corticosurrenali

di

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Numerosi ormoni steroidi sono stati isolati dalla corteccia surrenale in quantità sufficiente per il loro studio sperimentale e clinico; di una gran parte di essi si conoscono le proprietà fisiologiche e farmacologiche. Allo stato attuale delle conoscenze si deve ammettere che nella corteccia surrenale siano prodotti almeno tre gruppi di ormoni con proprietà fisiologiche diverse. Un primo gruppo è rappresentato da composti non ossigenati in posizione 11, attivi nel mantenere in vita l'animale surrenectomizzato e specialmente deputati alla regolazione del metabolismo idrico e salino: il desossicorticosterone è la sostanza meglio nota e più attiva di questo gruppo. Un secondo gruppo è rappresentato dai corticoidi ossigenati in posizione 11, che hanno un'azione elettiva sul metabolismo glicidico e protidico, mentre sono poco attivi nel mantenere in vita l'animale privato dei surreni e nella regolazione degli elettroliti: il composto più rappresentativo è il composto ,,E'' di KENDALL o Cortisone (11-deidro-17-idrossicorticosterone), meno attivi sono i composti ,,A'' (11-deidrocorticosterone), ,,B'' (corticosterone) ed ,,F'' (17-deidrocorticosterone). Un terzo gruppo infine comprende steroidi corticali ad azione genitale maschile e femminile. In un recente lavoro sperimentale ANTOPOL (1), in topini iniettati per diverso tempo con forti dosi di Cortisone ha notato la comparsa di noduli granulomatosi nel fegato, reni e milza, da cui isolò il *Corynebacterium pseudotuberculosis*; su trentasei animali iniettati giornalmente con dosi di mg. 1,25—2,5 dello steroide per un tempo variabile da 2 a 17 giorni, in otto si è avuto comparsa della malattia spontanea, mentre nei controlli non si è mai osservato segno alcuno di infezione in atto.

Nostri esperimenti, non ancora pubblicati, eseguiti su ratti iniettati giornalmente con mg. 5 di Cortisone per la durata complessiva di 20 giorni, hanno permesso di rilevare in 6 animali su 10 la comparsa di noduli granulomatosi di tipo pseudotubercolare sia nel polmone che nella milza (2); in altri esperimenti su ratti con ,,rene endocrino'' secondo SELYE e STONE, alcuni animali trattati anche per breve tempo con mg. 10 di Cortisone al giorno hanno fornito il reperto di una granulomatosi pseudotubercolare diffusa, con localizzazioni multiple polmonari, epatiche e cardiache (3). All'opposto, negli stessi esperimenti, animali di controllo ed animali trattati con dosi anche elevate di desossicorticosterone e di 21-acetossipregnenolone, non hanno mai presentato sviluppo spontaneo di infezione [1]). Nella letteratura più recente sulle proprietà biologiche del Cortisone viene inoltre avanzata l'ipotesi che in animali ed in uomini sottoposti a trattamento con questo ormone si possa avere un risveglio ed una generalizzazione di infezioni tubercolari; una semplice infezione latente potrebbe sfociare in una malattia ben costituita ed una infezione in atto potrebbe generalizzarsi.

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[1]) Anche SELYE (1950) ricorda necrosi, emorragie e ascessi polmonari in ratti sottoposti a forti dosi di Cortisone; formazioni ascessuali ha riscontrato anche nei reni e talora nel fegato. Selye pensa ad una diffusione di foci infettivi normalmente circoscritti e latenti in seguito a deficiente incapsulamento fibroso (Selye H., Stress, Acta endocr., Montreal 1950).

Con la presente nota ci siamo proposti di studiare l'effetto del Cortisone e di altri steroidi ad azione corticoide sulla malattia sperimentale da *Coccidioides immitis* Stiles in Rixford et Gilchrist, 1896; lo studio è stato fatto in stretto riferimento alle forme tubercolari, tenute presenti le strette analogie morfologiche, immunitarie e biologiche che questa micosi sperimentale presenta con la malattia tubercolare.

Metodi.

Lo stipite di *Coccidioides immitis* Stiles usato è etichettato nella nostra micoteca sotto il numero 085 con l'indicazione: DE ALMEIDA 1934, caso Marquet (GRAZIOSI, Ist. Batt. Roma n. 293). Il fungo è stato coltivato su agar glucosato per 5 giorni in termostato a 37° C; la vegetazione colturale è stata ripresa in 10 cc. di soluzione fisiologica sterile e la sospensione iniettata sotto la cute dell'addome in 20 ratti di peso medio di g. 150, tenuti a dieta mista e nelle stesse condizioni sperimentali, in dose di cc. 1 per animale.

Gli animali sono stati divisi in 4 gruppi: 5 animali furono trattati con mg. 5 al giorno di Cortisone (acetato di 11—deidro—17 idrossicorticosterone) (Ciba) sottocute; 5 con DCA (acetato di 11—desossicorticosterone) (Farmitalia); 5 con Artisone (21—acetossipregnenolone) (Farmitalia); 5 animali, infine, di controllo furono iniettati giornalmente con la stessa quantità di solvente degli steroidi (cc. 0,5 di olio di arachidi). Il trattamento è stato iniziato tre giorni prima dell'inoculazione del fungo e continuato per 17 giorni successivi. Il 17° giorno dopo l'inoculazione del fungo gli animali sono stati sacrificati per decapitazione; oltre al rilievo del peso corporeo e dello stato degli organi interni, ciascun animale ha fornito sangue, prelevato dalla carotide, per i conteggi degli eosinofili e per la formula leucocitaria. Gli eosinofili furono contati in camera di BÜRKER, dopo essere stati diluiti nel liquido proposto da DÜNGER (4).

Alla zoopsia sono stati prelevati per gli esami istologici il granuloma locale nel punto di inoculazione del fungo, la milza ed i surreni, e quegli organi in cui era presente o si sospettava una localizzazione del fungo. Gli organi prima della fissazione vennero accuratamente pesati e il peso riferito a g. 100 del peso corporeo iniziale dell'animale.

Il materiale è stato fissato in formolo al 10 % e liquido di CARNOY; le sezioni in paraffina sono state colorate coi comuni metodi della tecnica istologica e col metodo HOTCHKISS all'acido periodico — leucofucsina (5); sezioni del surrene al congelatore sono state sottoposte a studio istochimico della loro componente lipidica (esame a luce polarizzata di sezioni non colorate, prima e dopo trattamento con acetone a freddo per 30'; dimostrazione del colesterolo secondo LIEBERMANN-SCHULTZ, reazione con digitonina, colorazione con Sudan III e Sudan nero, reazione plasmale).

Risultati.

Peso corporeo

Gli animali inoculati con *Coccidioides immitis*, non sottoposti a trattamento con steroidi e tenuti come controlli, hanno sopportato bene l'infezione e praticamente hanno conservato immutato il peso di partenza. Lo stesso si è verificato negli animali che sono stati trattati con DCA e Artisone.

Al contrario, gli animali sottoposti a trattamento con Cortisone hanno presentato una forte diminuzione del peso corporeo, fino al 60 % rispetto ai valori di partenza. Il dimagramento più spiccato si è avuto in quegli animali che all'esame autoptico presentarono una diffusione dell'infezione agli organi interni; in questi casi, in 10a—12a giornata di malattia, si è reso manifesta una rapida e subitanea diminuzione di peso che progressivamente si andò accentuando nei giorni successivi.

Reperti ematologici

Nella tabella Ia sono riassunti i valori medi degli eosinofili circolanti e della formula leucocitaria nei vari gruppi. Nei linfociti abbiamo raggruppato sia i piccoli che i grandi linfociti.

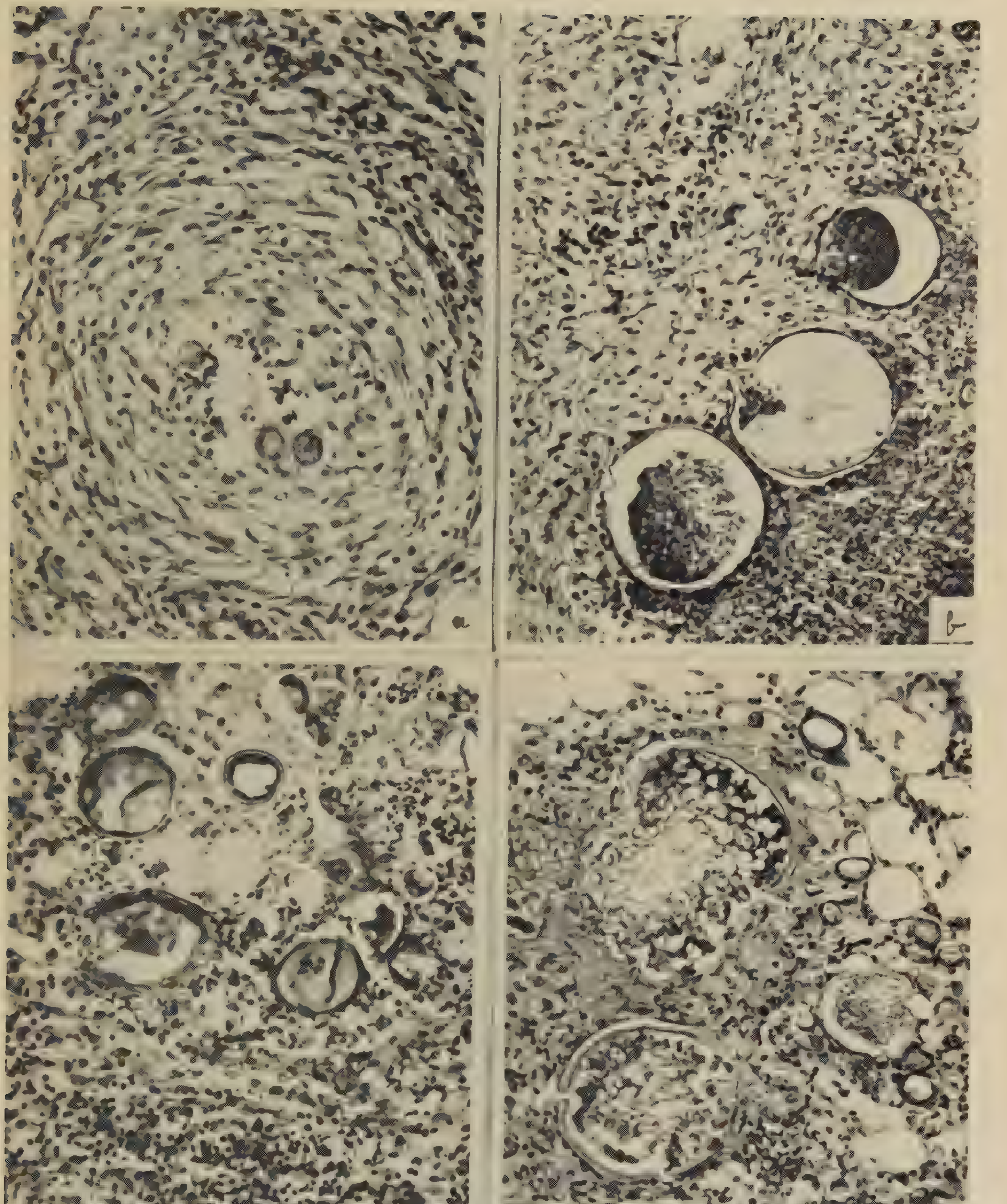

Fig. 1. Granuloma sottocutaneo da *Coccidioides immitis* Stiles (ingrandimento 310 d.)
a) ratto normale, granuloma tipico fibroblastico
b) ratto trattato con DCA, zoosporangi in zona di fibrosi
c) ratto trattato con Artisone, prevalenza di fenomeni essudativi
d) ratto trattato con Cortisone, zoosporangi maturi e necrosi.

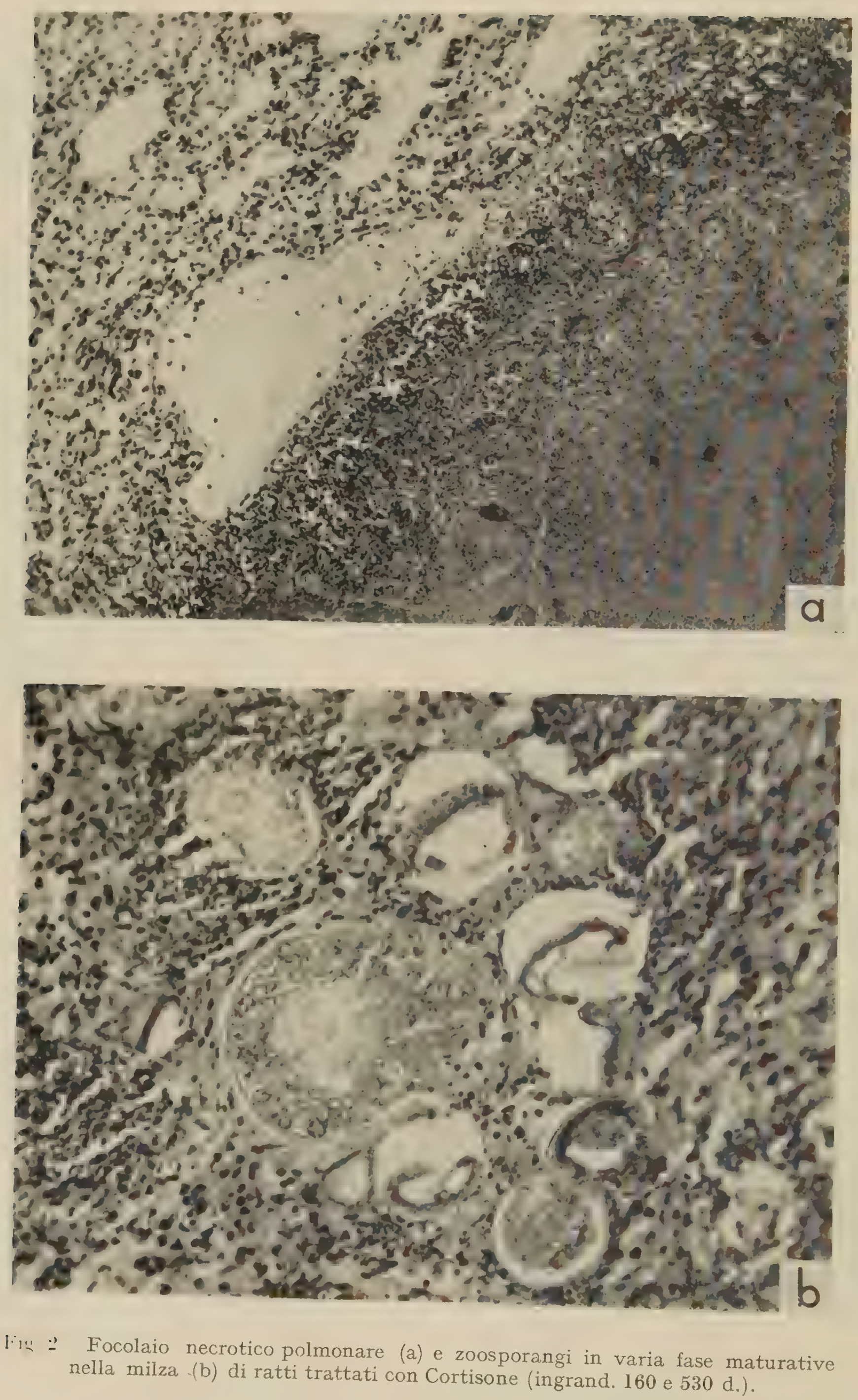

Fig 2 Focolaio necrotico polmonare (a) e zoosporangi in varia fase maturative nella milza (b) di ratti trattati con Cortisone (ingrand. 160 e 530 d.).

TABELLA 1a

Gruppo	Eosinofili mm³	Formula Leucocitaria %			
		Neutrofili	Eosinofili	Linfociti	Monociti
Controllo .	75 ± 21 ¹)	26,8	0,60	68,0	4,60
DCA . . .	117 ± 25	39,0	1,00	57,8	2,20
Artisone .	104 ± 52	23,8	1,80	72,8	1,60
Cortisone .	3 ± 1,6	66,0	0,25	30,5	3,25

¹) Errore standard calcolato secondo la formula $\sqrt{\frac{\Sigma d^2}{n\,(n-1)}}$

Come risulta dalla tabella, mentre nei controlli e negli animali trattati con DCA e Artisone il numero degli eosinofili e la formula leucocitaria non presentano evidenti deviazioni rispetto alla norma, in quelli trattati con Cortisone si è avuta invece una pratica scomparsa degli eosinofili in circolo ed una inversione del rapporto linfociti/neutrofili.

Reperti autoptici

Nei ratti controlli e in quelli trattati con DCA e Artisone l'inoculazione sottocutanea del fungo provocò lo sviluppo costante di un granuloma locale di dimensioni varie, ben delimitato rispetto ai tessuti vicini, di consistenza uniformemente dura e di aspetto bianco giallastro senza evidenza di aree necrotiche. In nessuno di questi animali si riscontrò una generalizzazione della forma morbosa; il processo rimase sempre circoscritto al punto di inoculazione e non si ebbero localizzazioni viscerali profonde.

Negli animali trattati con Cortisone i reperti locali e generali sono stati ben diversi da quelli riscontrati nei gruppi precedenti. Il granuloma locale presentava in generale dimensioni più limitate, consistenza molto ridotta, ampie zone necrotiche e scarsa delimitazione rispetto ai tessuti sani contigui. In 3 casi su 5 si ebbe una generalizzazione dell'infezione; un animale presentò un grosso focolaio nodulare compatto, con centro necrotico, che occupava gran parte del polmone destro; un altro analogamente offrì il reperto di una pneumonite nodulare granulomatosa necrotica con pleurite fibrinosa consensuale; un terzo infine, insieme a piccoli noduli giallastri in entrambi i polmoni presentò una localizzazione molto circoscritta in sede splenica. Negli stessi animali trattati con Cortisone si ebbe inoltre il noto reperto di una ipotrofia splenica e surrenale. Mentre nei controlli e negli animali trattati con DCA e Artisone il peso medio della milza e l'errore standard erano rispettivamente di mg. 716 ± 94,6, mg. 761 ± 16,3 e mg. 666 ± 80,9 per 100 g. ratto, il peso medio della milza dei ratti trattati con Cortisone era di mg. 284 ± 80,7, sempre per 100 g. ratto. Per quanto riguarda il peso dei surreni, nei controlli la media era mg. 32 ± 2,31, nei trattati con DCA e Artisone rispettivamente mg. 25 ± 1,94 e mg. 33 ± 3,17, mentre negli animali trattati con Cortisone si aveva un peso medio di mg. 17 ± 3,73 per 100 g. ratto.

I pesi splenici piuttosto elevati riscontrati nei diversi gruppi, a parte quello trattato con Cortisone, sono da mettere in rapporto con uno stato di congestione intensa dell'organo, particolarmente spiccata negli animali trattati con DCA. Anche il peso surrenale appare piuttosto elevato, probabilmente in rapporto allo stato infettivo; sempre lievemente più basso negli animali trattati con DCA ed enormemente diminuito nella media di quelli trattati con Cortisone.

Reperti istologici.

Negli animali di controllo l'inoculazione sottocutanea di *Coccidioides immitis* è stata seguita dalla comparsa di un tessuto di granulazione esuberante, con disposizione nodulare, ben circoscritto alla periferia da uno spesso strato fibroblastico. I singoli noduli granulomatosi constavano di una parte centrale necrotica in cui erano talora presenti delle zoospore del fungo in varia fase regressiva, insieme ad un certo numero di leucociti polimorfonucleati neutrofili. La zona centrale appariva circondata da un fitto alone di cellule epitelioidi e da giovani fibroblasti in attività fibrillogenetica. Gli elementi fibroblastici erano prevalenti in ogni sezione; essi presentavano un nucleo fusato più o meno ricco di cromatina ed un citoplasma abbondante, intensamente basofilo; i singoli elementi apparivano fittamente stipati con scarsa sostanza intercellulare interposta. Le aree necrotiche erano estremamente scarse e di poca estensione; il reperto fungino era oltremodo scarso, rappresentato da rare zoospore, variamente deformate e solo eccezionalmente da qualche zoosporangio. Cellule giganti, di tipo tubercolare o da corpo estraneo, erano presenti nei focolai in varia percentuale, in attività fagocitaria verso gli elementi del fungo. I reperti istologici offerti dagli animali trattati con DCA e Artisone non presentarono sostanziali differenze da quelli descritti; la reazione granulomatosa si è presentata però in genere meno attiva, più scarse le cellule giganti, insufficiente la produzione fibroblastica e spesso assente una netta delimitazione periferica dei focolai. Le aree necrotiche erano sempre più estese di quelle osservate nei controlli e nelle lesioni il fungo era presente in quantità maggiore. In questi animali e specialmente in quelli trattati con DCA, la componente essudativa polimorfocellulare predominava quasi sempre nel quadro istologico, con notevole partecipazione di polimorfi eosinofili. Il fungo appariva in forma di zoospore e anche di zoosporangi in diversa fase maturativa.

Negli animali trattati con Cortisone i reperti sono stati profondamente diversi. La produzione di fibroblasti, cellule epitelioidi e cellule giganti è mancata quasi totalmente: i pochi fibroblasti che si sono osservati a livello delle lesioni erano poverissimi di citoplasma, si disponevano in modo irregolare e non producevano fibrille. La lesione era priva di quella reazione demarcante periferica che è stata riscontrata costantemente nei gruppi precedenti. L'infiammazione non ha più presentato il carattere granulomatoso; ma ha assunto un tipo essudativo necrotico diffuso, nettamente differente. Ampie zone di necrosi omogenea con ampia partecipazione di leucociti polimorfonucleati neutrofili si sono riscontrate in ogni parte; nell'ambito delle necrosi e dell' infiltrazione polimorfocellulare si sono osservati numerosissimi zoosporangi in diversa fase maturativa; in tutti i casi il reperto del fungo è stato oltremodo abbondante. Nelle localizzazioni viscerali, polmonari e spleniche l'infiammazione ha conservato il tipo essudativo necrotico del granuloma sottocutaneo; nella milza e nel polmone, al centro o alla periferia delle lesioni, era presente il fungo in discreta quantità in forma di zoospore e zoosporangi.

Reperti istochimici

L'applicazione del metodo all'acido periodico — lanofucsina allo studio dei mucopolisaccaridi a livello delle lesioni infiammatorie provocate dal fungo non ha fornito reperti dimostrativi circa una eventuale riduzione di sostanza fondamentale mucoide intercellulare negli animali trattati con steroidi ed in particolare con Cortisone. A questo proposito il granuloma fungino nei diversi gruppi sperimentati ha fornito reperti praticamente identici; un particolare interessante è dato dalla notevole positività della reazione presentata sia dalle spore del fungo che dalle capsule degli sporangi, indice probabile di una singolare ricchezza in mucopolisaccaridi degli elementi fungini. Reperti molto dimostrativi si sono avuti dallo studio istochimico dei surreni nei diversi gruppi sperimentati. I reperti a questo proposito sono stati del tutto uniformi nel senso di una forte riduzione fino a pratica scomparsa delle reazioni del colesterole e colesteridi sia a livello della zona fascicolata che di quella glomerulare nei ratti a trattamento Cortisonico e nel senso di una netta riduzione, non però del grado osservato nel gruppo precedente, negli animali trattati con DCA e Artisone, mentre nel gruppo degli animali controllo le reazioni conservavano praticamente l'intensità normale.

Considerazioni generali

Il *Coccidioide immitis* Stiles, agente eziologico della coccidioidomicosi e della granulomatosi coccidioide, è un fungo parassita obbligato a doppio ciclo vitale, uno vegetativo ed uno parassitario. Inoculato nell'animale in forma di clamidospore, esso vegeta e produce zoosporangi provvisti di capsula in cui maturano le spore del fungo; queste (zoospore) vengono messe in libertà in seguito a rottura della capsula ed evolvono a loro volta in zoosporangi. Le notevoli affinità cliniche e biologiche che avvicinano la granulomatosi coccidioide ad altre granulomatosi, quale quella tubercolare, rendono di estremo interesse lo studio di questa rara malattia fungina. L'infiammazione provocata dal fungo, in rapporto con la recettività individuale e la virulenza del ceppo, può assumere il tipo granulomatoso puro oppure un tipo essudativo necrotico; l'infezione può restare circoscritta al punto di inoculazione, oppure generalizzarsi. La granulomatosi coccidioide è indubbiamente una malattia allergizzante ad equilibri molto complessi, analoga anche per questo, alla granulamatosi tubercolare (6—7).

Lo stipite di *Coccidioides immitis* da noi inoculato ha determinato nel sottocute del ratto una produzione locale di tessuto di granulazione iperplastico, ricco di fibroblasti e cellule giganti che ha bloccato l'infezione nel punto d'innesto. Negli animali che prima e durante l'infezione sono stati trattati con forti dosi di DCA e Artisone, steroli ad azione corticoide e proprietà prevalentemente mineraloattiva, la lesione provocata localmente dal fungo ha assunto come nei controlli il tipo granulomatoso iperplastico, con una certa maggiore estensione tuttavia di fenomeni essudativo-necrotici ed una certa maggior vegetazione del fungo in forma di zoospore e zoosporangi.

I reperti più salienti sono stati quelli degli animali trattati con Cortisone. In questi erano presenti i ben noti segni dell'effetto cortisonico: forte diminuzione del peso corporeo, scomparsa degli eosinofili nel sangue periferico, linfocitopenia, ipotrofia spleno-timo-linfatica, ipotrofia surrenale con scomparsa quasi completa delle reazioni istochimiche degli steroli a livello della sostanze corticale. Nel punto di innesto del fungo si è avuta una manifestazione essudativo-necrotica, è mancata la produzione di tessuto di granulazione specifico, è stata insufficiente la reazione demarcante del processo. Il fungo era presente in quantità enorme nelle lesioni. Insieme alla tendenza essudativo-necrotica della lesione locale ed alla abbondante proliferazione del fungo si è avuta in questi animali una generalizzazione della forma morbosa che è stata accertata in un'alta percentuale di casi, mentre essa è sempre mancata negli altri gruppi sperimentali. I polmoni e la milza sono le sedi di elezione per le localizzazioni viscerali del *Coccidioides immitis.*

Per l'interpretazione dei nostri reperti diverse ipotesi possono venire avanzate. E' noto attraverso ricerche di altri Autori (8) e nostre (9) che il Cortisone, forse per il grave impulso catabolico sul ricambio proteico, inibisce i normali processi di granulazione dei tessuti. Quest'effetto inhibitore si esercita soprattutto sulla produzione di fibroblasti che negli animali trattati con Cortisone appaiono in numero insufficiente, poveri di citoplasma, scarsamente basofili e con ridottissima tendenza a formare fibrille collagene. Nei nostri esperimenti la sostanza fondamentale mucoide interposta tra fibroblasti e fibrille non presenta differenze sostanziali nei diversi gruppi. La mancanza di una barriera istiale valida può spiegare in gran parte la generalizzazione dell'infezione fungina: ma altri possono essere ancora i fattori patogenetici di questa diffusione. Vogliamo alludere ai rapporti fra ormoni corticosurrenali, in particolare quelli ossigenati in posizione 11, ai quali appartiene il Cortisone, ed i fenomeni allergici, problema complesso ed ancora non definitivamente delucidato.

Da diversi Autori è stata prospettata l'ipotesi che estratti corticali ed ormone adrenocorticotropo ipofisario provochino nell'animale sensibilizzato un forte aumento degli anticorpi circolanti, fenomeno che si accompagnerebbe alla distruzione di tessuto linfatico ed ad aumento di gamma-globuline (10); Secondo DOUGHERTY e WHITE (11) e HAMMOND e NOVAK (12) l'estratto surrenale può anche condizionare una reazione immunitaria anamnestica. Ma altri riferiscono risultati contrari ai precedenti: secondo FISCHEL, LE MAY e KABAT (13) e secondo EISEN e STOERK (14) l'ACTH e l'estratto corticosurrenale non determinano aumento nel titolo degli anticorpi ematici pur provocando linfocitopenia; anzi il tenore in globuline anticorpali di conigli immunizzati verso il pneumococco verrebbe ridotto significativamente dal trattamento con ACTH o con Cortisone (BJORNBOE (15) e STOERK (16)), e la recente esperienza clinica ha dimostrato che forti dosi di Cortisone e ACTH diminuiscono l'aumentato tasso plasmatico di globulina (in particolare di gamma globuline) caratteristico di determinate forme morbose. Per quanto si riferisce alle manifestazioni scatenate dall'incontro antigene-anticorpo, sebbene sia da tempo noto che estratti corticali aumentano la resistenza degli animali surrenectomizzati allo shock anafilattico, sembra che su animali intatti non svolgano alcuna azione protettiva.

Lo shock anafilattico (FISCHEL (17), LEGER e coll. (18), HARRIS S. e HARRIS T. (19)), il fenomeno di Arthus (FISCHEL (17), HARRIS S. e HARRIS T. (19)), e la nefrite nefrotossica (KNOWLTON (20)) non vengono inibiti dall' ACTH o dal Cortisone.

Sembra invece che l'allergia batterica venga modificata dal trattamento cortisonico: secondo LONG e MILES (21), la reazione tubercolinica viene inibita nella cavia infettata con BCG; HARRIS S. e HARRIS T. (19) ottengono insensibilità locale e generale alla tubercolina in cavie e conigli infettati con BCG; FISCHEL (17) presenta il caso di un paziente trattato con forti dosi di ACTH, nel quale scomparve la iperreattività tubercolinica. Da notare però che le dosi di ACTH o di Cortisone necessarie per inibire la reattività tubercolinica sono così alte che FISCHEL suggerisce l'ipotesi che, accanto ad un'azione sui fenomeni immunitari, il Cortisone possa influenzare la risposta tubercolinica modificando localmente la reazione degli elementi connettivali secondo quanto prospettato da RAGAN (22).

Concludendo, l'evoluzione essudativo-necrotica e la facile generalizzazione dell'infezione da *Coccidioides immitis* in seguito a trattamento degli animali con Cortisone va quindi verosimilmente riportata a diversi fattori: in primo luogo come fattore più importante sta l'insufficiente reazione demarcante nel punto di innesto del fungo, poi la probabile caduta delle difese generiche antiinfettive allergiche ed immunitarie, e l'effetto aspecifico del Cortisone sul metabolismo generale dell'animale (dimagramento, bilancio azotato negativo).

Altri steroidi in studio, DCA e Artisone, non hanno modificato sensibilmente il decorso e l'aspetto morfologico dell'infezione; si deve quindi ritenere che nell'ambito degli steroidi corticali gli effetti riscontrati siano specifici di quegli ormoni che, come il Cortisone, presentano prevalentemente proprietà glicoprotido-attiva.

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Riassunto

Ratti iniettati sottocute con sospensioni di *Coccidioides immitis* Stiles hanno presentato localmente lo sviluppo di un granuloma fibroblastico gigantocellulare e nessuna tendenza alla generalizzazione dell'infezione. Il trattamento con DCA o Artisone non ha modificato sensibilmente il decorso e le caratteristiche istologiche dell'infezione. All'opposto il trattamento con Cortisone ha condizionato in un'alta percentuale dei casi una diffusione viscerale dell'infezione stessa; nella sede d'impianto del fungo la reazione dei tessuti, anzichè il tipo granulomatoso abituale, ha assunto un tipo necrotico-

essudativo, senza barriera demarcante fibroblastica e con intensa vegetazione fungina. Questi reperti vengono messi in rapporto con le proprietà antiallergiche ed antiimmunitarie generiche del Cortisone e con l'effetto catabolico sul ricambio proteico unitamente al noto effetto inibitore sui normali processi di granulazione dei tessuti esercitato da questo ormone.

Summary

After the subcutaneous injection of *Coccidioides immitis* Stiles, twenty rats showed a giant cell, fibroblastic granuloma; no general infection was observed. Treatment with DCA or Artisone did not modify the course and the histological pattern of the infection. On the contrary, in rats treated with Cortisone the infection was more severe, the subcutaneous granuloma showed larger necrotic and essudative foci, no fibroblastic tissue was formed, the fungous growth was conspicuous and visceral lesions were observed. These results are in agreement with the inhibitory effect of Cortisone on the allergic and immune responses and with the catabolic effect of the hormone on protein metabolism.

The use of bloodagar for the primary isolation of Dermatophytes

by

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(1.2.1951)

The relatively low percentage of positive cultures from fungus infections of the glabrous skin, in comparison with the results of microscopical examinations from such infections, is clear from the papers of various authors. (1, 2, 3, 4). As stressed elsewhere this common difficulty is encountered in attempted isolations from all types of mycotic infections of the skin, but is more pronounced in isolation from "tinea circinata". In this laboratory attempts have been made to increase the percentage of positive cultures from all types of fungus infections. Several methods have been tried, such as the inclusion of glucose agar, or Sabouraud's Honey agar media in the routine isolation procedures. These media gave no higher incidence of positive cultures than the routine Sabouraud's maltose agar.

In this paper there is presented a description of a method which has been proved successful in increasing the percentage of positive cultures from a certain clinical entity. The material used was derived exclusively from cases of the localized type of tinea circinata ("herpes tonsurans"). This clinical entity was chosen because no agent, other than fungi is known to cause tinea circinata.

Methods

The material from the lesions was scraped, without previous treatment of the site, into sterile Petri dishes or onto sheets of clean white paper. This material was used for microscopic examination in 30 % KOH and for repeated culture attempts. Sabouraud maltose agar slants were inoculated with small particles of the material at three different points and incubated at 24—26° C. If growth of pathogenic fungi failed to appear after 2 to 4 weeks, another slant was inoculated and if growth again failed to appear a third tube was inoculated. After three negative attempts the material was discarded. (One tube per specimen was used at each attempt.) During the first period covered by this survey (the first 59 cases), the medium was composed of the original french "Maltose brute" and "Pepton granule", and made up according to Sabouraud's original description. After the original materials were no longer available, a pure maltose (Difco) was tried for a short period, and then Sabouraud's maltose agar (Difco) was introduced permanently as routine medium. The composition of the blood agar was as follows: Stock culture agar (Difco) with the addition of 5 % whole human blood. In order to facilitate the quick differentiation of two of the cultured dermatophytes the hanging drop culture method with van Tieghem cells was used; the medium consisted of a 2 % glucose-lactose broth. The inoculum consisted of a small particle taken from a slant culture.

Results

As can be seen from table 1, the percentage of positive cultures from "tinea circinata" during a period of seven years was 40 %. The comparative investigation, using the additional blood agar medium was conducted during the last few years. *M. fulvum* was grown for the first time in this country from a lesion which clinically offered no characteristic which differentiated it from lesions caused by other fungus species. In only a small number of cases the inoculation of routine medium and blood agar was made at the same time. In most of the 24 microscopically positive cases the blood agar was inoculated after the routine medium failed to yield a positive culture. This accounts for the low number of positive cultures with the use of the routine medium in this

TABLE I. *Cultural results from* 100 *cases of tinea circinata; first series.*

No of specimens	Microscop. pos.	Culture results								Culture positive	
		T. gypseum	E. rubrum	T. violaceum	T. accuminatum group	M. fulvum	A. schönleini	unidntified T.	No growth	total	%
100[1]	80	14	6	13	3	1	1	2	60	40	

Medium: SABOURAUD's Maltose agar slants prepared with original french chemicals, pure maltose (Difco) and dehydrated Difco medium.

TABLE II. *Comparative culture results from* 24 *cases of tinea circinata; second series.*

No. of specimens	Microscop. pos.	Medium	Culture results						positive cultures	
			T. gypseum	E. rubrum	T. violaceum	T. accuminat group	M. fulvum	no growth	total	%
24	24	Sab. malt.	—	1	2	1	1	19	5	20
		Blood agar	1	4	7	1	1	10	14	60

Medium: SABOURAUD's maltoseagar dehydrated (Difco), and Blood agar.

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[1] A number of these specimens were included in an earlier paper from this department (8).

series of experiments (table II). The blood agar yielded a positive culture in 15 cases which on the routine medium failed to grow, table II. Even if the number of positive cultures on the blood medium is low compared with the number of positive cultures on the routine medium in the first series (table I) the increase in yield with the use of blood agar is 20 %.

Comment

The reason for the relatively low yield of positive cultures from definitely mycotic lesions is not known, but it may be caused by a deficiency in certain fungus strains which does not allow them to utilize relatively simple media without the addition of some essential growth factor. This suggestion seems to be in line with the observation of FOWLE and GEORG (5) who noticed that a certain number of faviform organisms grew on primary isolation only with the addition of blood or thiamine to ordinary media; and of GEORG (6) in her experiments with a number of different strains of *T. violaceum*. The same fact seems to be borne out by the study of LANGERON and MILOCHEVITCH (7) introducing the so called material of polysaccharide bases. Because of these reasons blood agar was introduced when material from "tinea circinata" failed to grow on SABOURAUD'S maltose agar. For identification purposes the cultures grown on blood agar had to be transferred onto SABOURAUD'S maltose agar. The incidence of the various fungi grown from "tinea circinata" shows that *T. gypseum* (*mentagrophytes*) and *T. violaceum* occur to a much greater extent than *T. rubrum*, whereas the *T. crateriforme* group, *A. schoenleini* and *M. fulvum* seem to be quite uncommon. Although the number of cultures in table II, second series, is quite small in comparison with the number of the first series, table I, it is interesting to note that the organism which chiefly benefited by the addition of blood agar is *T. violaceum*. Whether, however, this beneficial effect of the addition of blood to the agar is due solely to its vitamin content is open to doubt. PEPTONES used in media are known to contain appreciable amounts of water soluble vitamins of the B group. It is conceivable that some other constituent of blood accounts for the above mentioned effects and further work on this problem will be done in the future.

Some difficulties were encountered in differentiating downy colonies of *T. gypseum* from *T. rubrum*, especially if the latter organism failed to produce the characteristic pigment. Due to the long, prevailingly dry summer season, the tube content frequently dried up before pigment production could take place. Therefore, in order to facilitate the distinction between these two organisms in as short a time as possible, the hanging drop culture method was used. Often, after only three to four days incubation the characteristic fructification organs could be seen distinctly. *T. gypseum* usually develops spores in clusters, the spores being predominantly round to clavate, and plump typical microconidia. In most cases spirals were formed on the glucose-lactose medium. The latter structure was entirely absent with *T. rubrum*, the spore being predominantly pyriform, elongated and mostly arranged in regular distances on both sides of long straight hyphae the socalled "Hyphes sporiferees". The spindles are narrow, long with blunt ends, and a few compartments, and develop mostly in an "intercalary" position. These distinctive features used in identification apparently account for the change in the ratio of *T. rubrum* against *T. gypseum* in favour of *T. rubrum*. It seems that before the introduction of this method a number of downy cultures of *T. gypseum* should have properly been identified as *T. rubrum*.

Summary

The introduction of blood agar as additional medium for primary culturing of fungi from "tinea circinata" lesions has proven successful in increasing the yield of positive results. Early distinctive features of *T. gypseum* and *T. rubrum* in hanging drop cultures are described.

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Schedae Mycologicae I-XI

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I. Meliola (*Chaetomeliola*) javanica Cif., n.sp. (Fig. 1)

Coloniis nigris, epiphyllis, sub-crustosis, plus vel minus regulariter rotundatis usque sub-orbicularibus, singulis, isolatis vel sub-confluentibus, sed distinctis, 1—3 mm. diam.; mycelio ex byphis opposite ramosis, symmetricis, rarius anastomosantibus, rectis vel sub-sinuosis, flavidulis usque flavo-brunneis, regulariter septatis, 6—8 μ. crassis composito; hyphopodiis capitatis numerosis, regulariter distributis, typice oppositis, non vel rarius alternantibus aut unilateralibus, brunneis, inter se separatis, cellula apicale ovoidea, oblonga vel ellipsoidea, usque sphaeroidea, simplici, recta, rarius curvula aut perpendiculariter inserta, 7—11 × 6—9 μ., vel 6—10 μ. diam., cellula basilare variabile sed manifesta, 3—10 μ. longa, concolore; hyphopodiis mucronatis non visis; setis myceliaribus irregulariter distributis, mox non praesentibus vel rariis, mox numerosis, typice acuminatis usque acutis, rarius obtusis vel

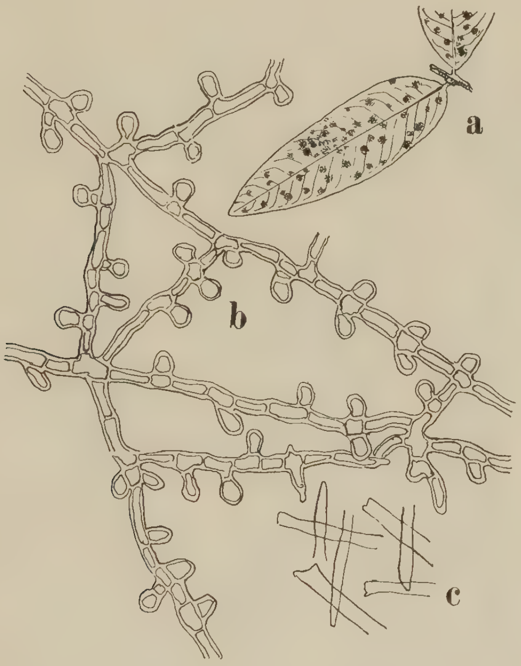

Fig. 1. *Meliola javanica* n.sp, *a*) Habitus of the fungus; *b*) mycelium with capitate hyphopodia; *c*) setae.

truncatis, nigris, rectis, sub-erectis, non fasciculatis sed simplicibus singulisque (rarissime aggregatis usque sub-fasciculatis), base geniculata, 150—200 × 7—11 μ.; setis perithecialibus similaribus sed minoribus, 75—150 μ. longis, typice acutatis vel acuminatis, singulis usque dense aggregatis, sed non fasciculatis, prope margine peritheciorum locatis; perithecia sparsa, singula vel sub-aggregata, nigra usque brunneolo-nigra, opaca, sphaerica, prope margine dentata, indistincte celluloso-polygonata, 115—190 μ. diam.; ascis 2—6-sporis; ascosporis brunneis usque brunneo-fuscis, ellipticis vel cylindraceis, apicibus rotundatis, 4-septatis, ad septa constrictis, 36—40 × 16—20 μ.; paraphysioides mucosa, indistincta.

HAB.: in foliis *Cassiae siameae*, Archipel. Ind., Insula Java, Mampar, leg. BAKHUIZEN V. D. BRINDLE (?) X. 1922, mis. Hort. Bot. Bogoriensis, N. 2342.

Formula, secondo STEVENS: 3413.3121. Questa specie è certamente diversa dalla *M. acrotricha* Syd. (formula: 3413.4222), sopratutto per il fatto che è, sino ad un certo punto, intermediaria tra i sottogeneri *Eumeliola* e *Chaetomeliola*, in quanto le setole periteciali non sono sempre presenti e regolarmente distribuite, trovandosi talvolta alla periferia dei periteci. Vi sono anche altre diversità morfologiche oltre quelle inerenti alla pianta ospite parassitata. Tali setole periteciali si vedono anche, talvolta, sui nodi micelici formati da ife radiato-anastomizzate, primi abbozzi di formazione dei periteci.

II. Meliola ingae Stev. et Teh. var. adenantherae Cif. var. nova. (Fig. 2)

Coloniis punctiformibus, sub-pulvinatis, rotundatis vel orbicularibus, dense aggregatis sed irregulariter distributis, nigris, opacis usque velutinis, mox isolatis singulisque, haud confluentibus, epiphyllis, circ. 0,5 mm. diam.; mycelio ex hyphis biformis aliquando delicatis, brunneis usque fusco-flavis, septatis, interdum crassiusculis, brunneo-fuscis usque nigridulis, indistincte septatis, composito, hypharum segmentis irregulariter sinuosis usque sigmoideis; hyphopodiis capitatis saepe alternantibus vel unilateraliter dispositis, rarius oppositis; setis perithecialibus paucis, molliusculis brunneisque, vel rigidis nigrisque, apice obtusato usque acuminato, 125—200 μ. longis; peritheciis

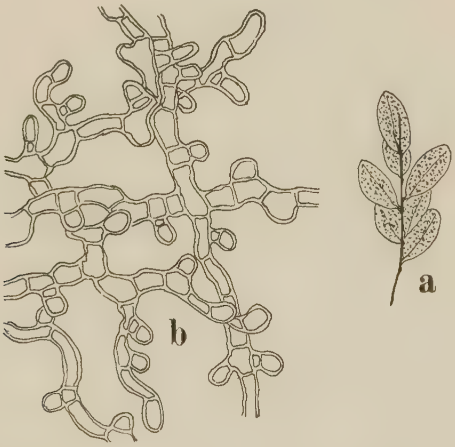

Fig. 2. *Meliola ingae* Stev. et Teh. var. *adenantherae* var. nova. *a*) Habitus of the fungus; *b*) mycelium with capitate hyphopodia.

prope marginem setosis, brunneo-fuscis, distinctissime cellulatis, sub-pellucidis, 200 μ. diam. vel minus; ascis 4—8-sporis; ascosporis uniformibus vel irregularibus, 38—43 × 15—18 μ.

HAB.: in foliis *Adenantherae microspermae*, Archipel. Ind., Insula Java, Tjipetir, leg. MAAS, 1928, mis. Hort. Bot. Bogoriensis, N. 7370.

Formula, secondo STEVENS: 3403.4220. Questa varietà è differente dal tipo, secondo la descrizione di STEVENS e TEHON (Mycol., Vol. XVIII, pag. 20.1926), trovato su *Inga* nella Guiana inglese, che ricorda per varie caratteristiche incluso l'habitus. Benchè sia da riferire al sottogenere *Irene* (Theiss. et Syd. emend. Stev.) Cif., la sua posizione sistematica non è del tutto sicura, poichè le setole sono rarissime, e frequentemente pure localizzate sui nodi micelici. Si possono pure vedere degli ifopodi capitati isolati, portati all'estremità di una ifa micelica più o meno lunga.

III. Meliola (*Irenina*) Boedijnii Cif., n.sp. e Arthrobotryum penicillium (Speg.) Stev. (Fig. 3)

Coloniis nigris, crustosis vel sub-crustosis, isolatis, singulis sparsisque, vel, rarius, aggregatis, sed non confluentibus, 0—1,5 mm. diam., epiphyllis; mycelio ex hyphis consuete oppositis constituto, segmentis hypharum rectis vel curvulis, brunneo-nigridulis, indistincte septatis, 10—12 μ. crassis; hyphopodiis capitatis pro more oppositis, rarius alternantibus vel unilateralibus, irregulariter distributis, saepe densissimis, aliquando laxe dispositis, brunneo-fuscis, 18—29 μ. longis, cellula superiore sphaerica vel sphaeroidea aut sub-oblongata, rarius elliptica, integra (rarissime sub-lobata), recta vel curvula, 14—18 μ. diam. vel 16—22 × 12—16 μ., cellula inferiore 3—6 μ. longa, plus vel minus

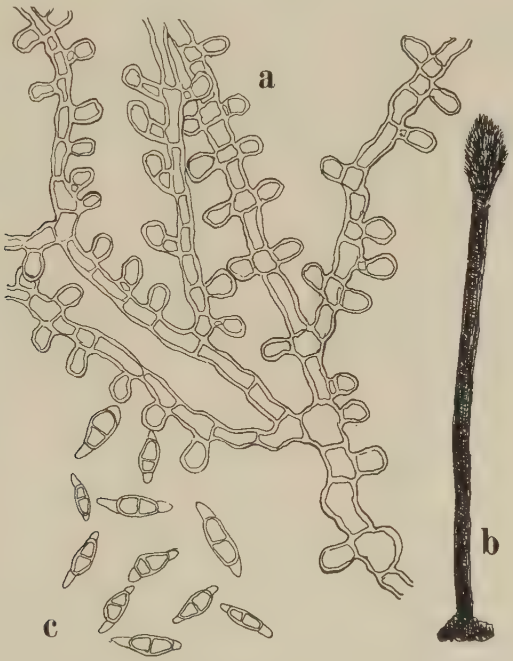

Fig. 3. *Meliola Boedijnii* n.sp. *a*) Mycelium with capitate hyphopodia. *Arthrobotryum penicillium* (Speg.) Stev. *b*) Synnema; *c*) Conidia.

indistincta; hyphopodiis mucronatis non visis; setis perithecialibus myceliaribusque nullis; perithecia pauca, sphaerica, sub-depressa, nigra, carbonacea vel carbonaceo-crustosa, 80—150 μ. diam., non aggregata sed isolata, singula; ascis 2—4-sporis; ascosporis ellipticis vel ovoideis, rarius cylindroideis, utrimque rotundatis, brunneis vel fusco-brunneis, 4-septatis, ad septa leniter constrictis, 40—50 × 18—22 μ.

HAB.: in foliis *Schefflerae aromaticae*, Archipel. Ind., Insula Java, Tjisaroea, leg. BOEDIJN (N. 767), VII. 1930, comm. Inst. Bot. Bogor., N. 11740.

La formula di questa specie, dedicata al micologo olandese Dr. BOEDIJN, raccoglitore del materiale studiato, è, secondo STEVENS: 3103.4320. E' la prima specie del sottogenere *Irenina* (Stevens) Cif. (*Meliola* senza setole o *Irene* Theiss. et Syd., p.p.), trovata su un membro della famiglia Araliaceae, ed avente gli ifopodi capitati opposti o unilaterali o alternati. Su piante della stessa famiglia sono state descritte *M.* (Irenina) *clerodendri* Stev., M. (Irenina) *morototoni* Speg. e *M.* (Irenina) *glabroides* Stev., tutte diverse dalla *M. Boedijnii.*

Il fungo è parassitato dall' *Arthrobotryum penicillium* (Speg.) Stev. Ivi i sinnemi sono eretti o suberetti, neri, poco allargati alla base e poco espansi alla cima, 725—1000 × 30—45 μ. I conidiofori sono individualmente differenziati soltanto all'apice del sinnema, di colore giallastro, sottili, 15—30 × 4—6 μ. semplici, retti, disposti a pennello, non denticolati, ottusi, nastriformi, apparentemente senza setti (almeno nella porzione libera); i conidi sono isolati e prodotti in cima ad ogni conidioforo, ovoidi od ellittici, più o meno rigonfiati nella parte centrale, talvolta a forma di clava, 3-settati trasversalmente, con le due cellule centrali di colore bruno chiaro e maggiori delle due cellule basali ed apicali, queste di colore giallo brunastro sino a sub-ialine; la cellula apicale e cortissima, da sub-conoide a sub-acuta, di color giallo o giallo brunastro; la cellula basale è più lunga ma stretta, sino a sub-caudata e raramente sino a caudata, giallastra o sub-ialina. Complessivamente i conidi misurano 28—34 × 10—13 μ.

IV. **Meliola Hercules v. Höhn.** (Fig. 4)

Un esemplare di questa specie è stato inviato dal Giardino Botanico di Buitenzorg (su foglie di *Saccharum spontaneum*, Archip. Ind., Insula Java, Tjigombong, leg. BOEDIJN (N. 761), VIII. 1930. Coll. Ist. Bot. Bogor. N. 11737).

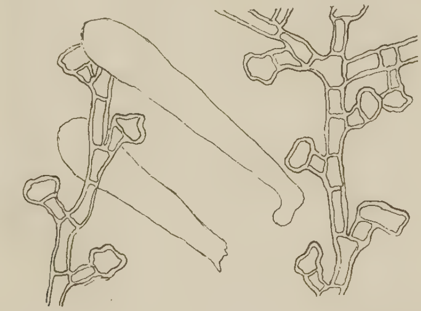

Fig. 4. *Meliola Hercules* v. Höhn. Mycelium with hyphopodia and setae.

Il fungo forma delle piccole colonie nerastre, diffuse sino a radiate, del tutto superficiali, di 2—3,5 mm. diam.; le ascospore sono un poco più grosse che negli esemplari tipici (42—46 × 18—23 μ.); le setole miceliche sono scarse, corte (circa 100 m. di lunghezza), molto ispessite, rigide, e a forma di clava.

La specie è stata descritta da von Hoehnel (Sitzber. Akad. Wiss. Wien, Math.-Nat. Kl., Vol. CXVIII, pag. 316.1909) su una Graminacea ignota (forse *Anthistiria*) dell'isola di Giava. Nell'esemplare studiato, le setole sono molto simili ai giovani sinnemi di una Stilbacea Dematiacea, ad esempio, *Arthrobotryum*; verisimilmente si tratta solo di una fortuita analogia, in quanto non si è osservata una ulteriore evoluzione dei presunti sinnemi.

V. Meliola aegiphilae Stev., M. macarangae Syd. et M. mangiferae Earle (Fig. 5)

Il fungo giavanese vivente sulle foglie di *Clerodendron incisum* var. *macrosiphon* (Archip. Ind., Insula Java, Hortus Bogoriensis, leg. Boedijn (N. 757), VII. 1930, mis. Inst. Bot. Bogor., N. 11728) è quasi identico alla *Meliola aegiphilae* Stev. (Ann. Mycol., Vol. XXVI, pag. 208.1928) trovato su *Aegiphila* nella Guiana inglese e a Santo Domingo. Ciononostante si notano alcune differenze, benchè forse di valore secondario, per una certa analogia dell'esemplare giavannese con la *M. clerodendricola* Stev., indicata su *Clerodendron*. In effetti l'esemplare giavanese si accorda con la *M. aegiphilae* sopratutto per forma e dimensioni degli ifopodi capitati, forma e dimensioni delle setole miceliche (in generale ottuse, ma anche dentate sino a brevemente forcate). La differenza più importante tra *M. aegiphilae* e *M. clerodendricola* — e forse l'unica differenza — risiede nel fatto che nell'ultima specie le setole miceliche sono sempre semplici. L'esemplare su *Clerodendron incisum* var. *macrosiphon*, con le sue setole normalmente semplici, ma variabili sino a ramosette, è intermediario tra le due specie.

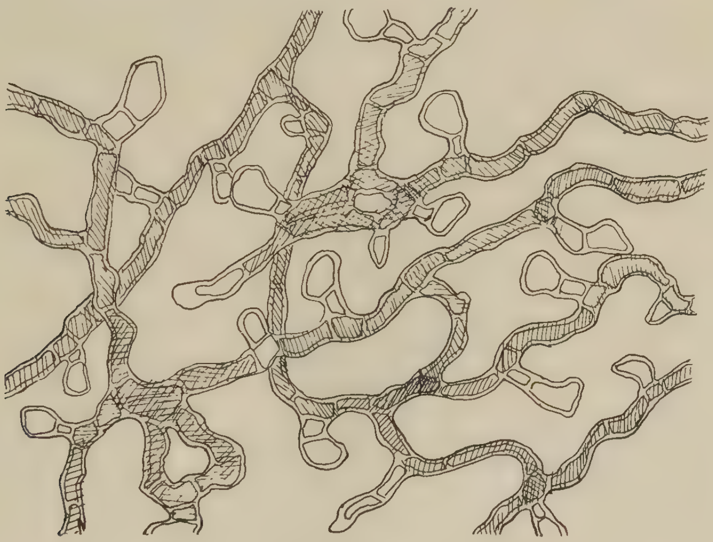

Fig. 5. *Meliola aegiphilae* Stev. (javan. spec.). Mycelium with hyphopodia.

L'esemplare di *M. macarangae* Syd. per il fungo vivente su *Macaranga triloba* (Arch. Ind., Insula Java, Tjilodong, leg. v. Overeem (N. 800), V. 1922, mis. Inst. Bot. Bogor.) s'accorda bene con la descrizione di questa specie: le setole sono di 290—600 μ. di lunghezza, e sub-ottuse sino ad acuminate all'estremità libera.

La *M. mangiferae* Earle (Bull. New York, Bot. Gard., Vol. III, pag. 307. 1905) su foglie di *Mangifera* sp. (Arch. Ind., Insula Java, Batavia, leg. Boedijn,

XII. 1928, mis. Inst. Bot. Buitenz., N. 7369) è identico alla specie americana su *Mangifera indica*. Le setole sono sempre semplici, raramente sub-dentate, mai veramente dentate o ramose. I periteci sono talvolta circondati da corte setole (lunghe 43—90 μ.), rette o curvate, nere, rigide. Le spore sono un poco più piccole che la media degli esemplari americani, ma sempre nel limite delle oscillazioni specifiche.

Questa quasi identità tra gli esemplari americani (nel cui territorio la *Mangifera indica* è stata importata dall'Asia tropicale) e quelli giavanesi, nella cui area probabilmente questa specie si è originata, è di notevole interesse biologico, poichè le Meliolineae generalmente si trovano solo nelle specie aborigene e nell'area aborigena; la *M. mangiferae* è una delle più conclamate eccezioni, in quanto estremamente comune nell'area americana di cultura del mango. Ciò indurrebbe a credere piuttosto ad una importazione della *Meliola* dall'area assiatica a quella americana che ad un adattamento nell'area americana di una *Meliola* passatavi da qualche altro ospite. Tutta la questione della specializzazione biologica delle Meliolae è ancora da affrontare.

XIII. Arthrobotruym Gorgoneum Cif., n. sp. (Fig. 6)

Coloniis melioloideis, nigris, velutinis, plus vel minus isolatis usque confluentibus, irregularibus; mycelio ex hyphis flavidulis usque brunneolis, dense sed irregulariter ramosis, copiosis, frequenter anastomosantibus, septatis, 4—6 μ. crassis composito; synnematibus simplicibus, gregariis vel sub-solitariis, densis, nigris, plus vel minus erectis, sursum expansis, base leviter incrassata

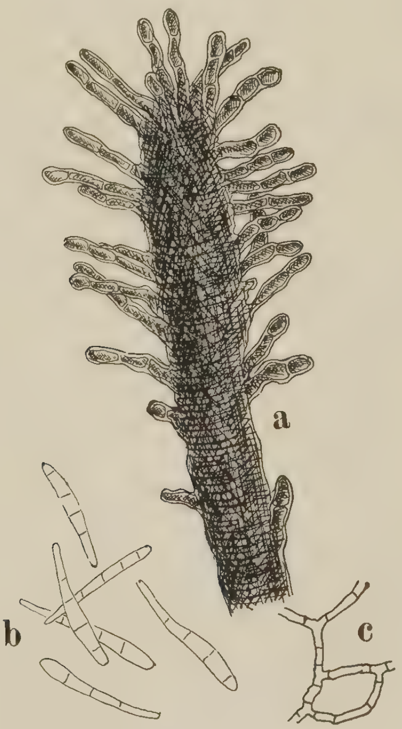

Fig. 6. *Arthrobotryum Gorgoneum* n.sp. *a*) Synnema; *b*) Conidia; *c*) Hyphae.

sed non inflata, ex 20—50 conidiophoris relaxate intertextis compositis, 400—700 μ. longis, base 75—80 μ. crassa, apice 48—60 μ.; conidiophoris aggregatis flavobrunneis vel brunneis, ad apicem distinctis, simplicibus, non ramosis, pro more curvulis vel sinuosis, rarius rectis, septatis, sursum sub-dentatis, ex cellulis 36—70 μ. longis compositis, in toto 400—750 μ. longis, 6—8 μ. crassis; conidiis singulis, saepe acro-pleurogenis, rarius acrogenis, rarissime pleurogenis, elongato-ellipsoideis vel ovato-elongatis, flavidulis, flavo-brunnei vel brunneolis, simplicibus usque sub-caudatis, prope apicem breviter rotundatis, base elongata vel elongato-caudata, acutata usque sub-obtusa, 3—4-septatis, pro more 4-septatis, ad septa non constrictis, 45—65 × 6.5—9 μ.

HAB.: parasiticus in *Meliolae* (Ireninae) *vilis* Syd., in foliis *Viticis bicoloris*, Archip. Ind., Insula Java, Sawangan, leg. BAKHUIZEN V. D. BRINK, N. 5764, X. 1922, mis. Ist. Bot. Bogoriensis, N. 2344.

Questa specie sembra diversa dalle altre specie di *Arthrobotryum* meliolicole (cfr. HANSFORD, I.M.I. Myc. Pap. N. 15, pag. 217.1946) per la disposizione caratteristica ,,a testa di Medusa'' delle estremità libere dei conidiofori, curvate o tortuose, formanti una sorte di testa aperta ed espansa; da ciò il nome specifico. Con le altre specie i cui conidi hanno in media 4 setti differisce per l'habitus e le diverse caratteristiche su indicate.

VII. Necraphidium Cif. n. gen.

Con il binomio di *Ramularia zoophila* Sacc. et Trott. (in Sacc., Bull. Orto Bot. Univ. Napoli, VI, p. 32. 1918) fu descritta una specie fungina su larve di Afidi (forse *Callipterus alni*) viventi su *Alnus glutinosa* ad Avellino.

Il riesame del materiale autentico dell'Erbario Saccardiano di Padova, ha mostrato che il fungo differisce da *Ramularia* non solo per vivere su insetti anzichè essere parassita di piante, ma anche per i conidiofori da lassamente fasciculosi sino a non fasciculati, singoli o sub-isolati, da sub-eretti a sub-repenti, spesso portanti dei rami di secondo ordine sterili e corti; per la presenza di micelio superficiale (qua e là ridotto e forse di secondaria sopravvenienza), ed infine per la forma dei conidi che ricorda — salvochè per il numero dei setti — quelli di un *Fusarium* o piuttosto di un *Fusoma* (nel senso classico saccardiano). Da *Fusoma* differisce però, oltrechè per le attitudini biologiche, per i conidi mai più che 1-settati, per la presenza ben manifesta di conidiofori, ecc.

NECRAPHIDIUM Cif. n. gen. — Zooparasiticus; mycelio superficiale parum evoluto; conidiophora laxe fasciculata usque singula, continua, simplicia vel brevissime 1-ramulosa (ramulis secundaris sterilibus), suberecta usque subrepentia; conidiis fusoideis, acrogenis, continuis vel 1-septatis, breviter catenulatis.

TYPUS: *N. zoophilum* (Sacc. et Trott.) Cif., n. comb. (= *Ramularia zoophila* Sacc. et Trott.).

VIII. Camptomeris robiniae (Shear) nob., n. comb. e C. acaciae (Syd.) nob., n. comb.

Sul *Fusicladium robiniae* Shear ha scritto, per il Wisconsin, GREENE (Amer. Midl. Nat., Vol. XXXIV, pag. 261.1945). Per quanto ci è stato possibile osservare nello scarso materiale studiato (F. colombiani, N. 1619) questa specie dovrebbe essere riferita al gen. *Camptomeris* Syd., di cui le varie specie oggi note appaiono specializzate su foglie di Leguminose arboree; tali *C. calliandrae* Syd., *C. leucaenae* (Stev. et Dalb.) Syd.; *C. desmanthi* Cif.

Probabilmente anche il *Septogloeum acaciae* Syd. (Am. Mycol., Vol. XIV, p. 220.1916) su *Acacia arabica* dell'India è *Camptomeris acaciae* (Syd.) nob., n. comb.

IX. Cercoseptoria sapindophila (Speg.) nob., n. comb.

HAB.: su foglie vive di *Serjania* sp., Venezuela, Est. Aragua, Ocumare de la Costa, in incultis, III.1948.

Questa specie è stata descritta in Puerto Rico da SPEGAZZINI (Ann. Mus. Nac. B. Aires, VI, 351.1899) come *Fusarium*? *sapindophilum* su una Sapindacea indeterminata, e ridescritto proprio in Venezuela da H. SYDOW (Ann. Mycol., XXVIII, pag. 223.1930) su *Serjania mexicana*, riportandovi pure il *Fusarium serjaniae* Syd. (Hedw., Vol. XL, pag. (2).1901) nel Messico su *S. racemosa*.

L'osservazione di un esemplare bene fruttificato ha confermato il dubbio espresso da H. SYDOW trattarsi piuttosto di una *Cercoseptoria* che di un *Fusarium*.

X. Infrafungus Cif., n. gen.

Con il binomio *Fusarium micropus* fu descritto da SACCARDO (Philip. Journ. Sci., Vol. XVIII, p. 605.1921) una specie parassita del *Cladosporium herbarum* su *Morus alba* in Cina, con l'annotazione espressa che la specie aberrava dal genere e doveva essere ulteriormente studiata.

Avendo da classificare un fungo simbionte o semiparassita del *Cladosporium fulvum*, per la cortesia del Prof. GOLA, ci fu possibile esaminare un frammento del materiale autentico del SACCARDO.

L'assenza completa di sporodochi o di aggregati sporodochiformi; i conidiofori molto corti e semplici, quasi dei rami appena specializzati derivanti dalla ife miceliche reptanti, ed eretti o sub-eretti, non o quasi non aggregati, ma sparsamente distribuiti in cespituli intimamente mescolati alle ife ed alle fruttificazioni del *Cladosporium* (ognuno dei conidiofori portando all'apice 1—2 conidi); lo scarsissimo ma non nullo micelio sterile, distinguono nettamente il fungo da *Fusarium*. La specie potrebbe meglio riferirsi ad una *Dactylella* nel senso di GROVE se il genere non sembrasse, per le specie meno recenti, limitato a forme saprofite e lignicole, senza micelio sterile, con conidiofori lunghi e bene differenziati dalle ife sterili. (Le specie parassite di Nematodi descritte, ad es., da DRECHSLER [Mycol., Vol. XXIX, pag. 487.1937] si allontanano in parte dal tipo del genere, e la specie vivente assieme al *Cladosporium* dovrebbe, se mai, essere comparata con la *Dactylella* fusarioide *D. leptospora*). Dal genere *Monacrosporium* per la sola specie vivente in consorzio con *Meliolae* (*M. meliolicola* Cif.) differisce nettamente in quanto quest'ultima specie ha dei conidiofori ben differenziati, e un abbondante micelio lanoso o aracnoideo, con conidi di solito 5-settati.

Il fungo in istudio potrebbe anche avere delle affinità con il genere *Amastigosporium* Bondarz.-Monteverde (Meth. Mikr. Ross., Vol. V, pag. 2.1921), sul quale, però, manchiamo di informazioni.

In attesa che si effettui una revisione del gruppo di generi *Dactylella, Monacrosporium, Fusoma, Menispora* ed altri affini, ci pare opportuno distinguere genericamente il fungo simbionte del *Cladosporium*:

INFRAFUNGUS Cif., n. gen. — Fungiculus, effusus, hyalinus; mycelio sterile parum evoluto; conidiophora parum differentiata, erecta, abbreviata, simplicia; conidia acrogena, solitaria vel binata, falcata, hyalina, 1—3-septata.

TYPUS: *I. micropus* (Sacc.) nob., n. comb. (= *Fusarium micropus* Sacc.).

XI. Millerburtonia Cif., n. gen. e Chalcosphaeria oyedeae (Syd.) nob., n. comb.

HAB.: in foliis *Oyedeae verbesinoides* parasitica, Venezuela, Est-Aragua, bosco attorno a Rancho Grande, 900 m. circa, VII. 1948.

Nulla abbiamo da aggiungere attorno alle caratteristiche di questa bella specie, descritta in Costa Rica da SYDOW (Ann. Mycol., Vol. XXIII, 353.1925) come *Phyllocalis oyedeae,* e quindi in Venezuela da CHARDON (Bul. Soc. Venez. Ci. Nat., V (40), 261.1939) come *Phomatospora oyedeae,* ed infine da MILLER e BURTON (Mycol. XXXV (1), 90.1943) riferita a *Hypospila oyedeae* (Syd.) Mill. et Burt. Ma le ragioni esposte da VON HÖHNEL (Ann. Mycol., XVI, 97.1918), circa i rapporti tra *Sphaeria pustula* Pers. e *Hypospila* Fries — che lo hanno condotto a creare il gen. *Chalcosphaeria* — sono tuttora valide. La riconvalidazione di *Hypospila pustula* (Pers. et Fr.) Karst. secondo CLEMENTS e SHEAR (Gen. of Fungi, II ed., pag. 271.1931) è inaccettabile se la stessa specie è assunta come tipo di *Chalcosphaeria*: oltre tutto è da tenere in conto il parassitismo e in generale il loro comportamento biologico.

Bene spesso, nelle stesse macule, si hanno loculi ascosporiferi e loculi conidiferi, e la forma conidica è stata descritta sia da CHARDON che da MILLER e BURTON, ma non nominata. La indichiamo dedicandola a J. H. MILLER e M. G. BURTON, studiosi di gruppi critici di Ascomiceti tropicali, come:

MILLERBURTONIA Cif., n. gen.— Foliicola, parasitica, maculigena; acervula subepidermica, dein erumpentia, minuta, ostiolata; conidiophora hyalina, erecta, stipata, simplicia vel late ramosa; conidia filiformia- aciculata, hyalina, continua, delicata.

Est st. conidioph. *Phyllocalidis* (Diaporthacearum). TYPUS: *M. oyedeae* Cif., n. sp. (St. ascoph. *Chalcopshaeriae oyedeae* (Syd.) nob., n. comb.).

Maculis amphigenis, decoloratis, 3—6 mm. diam., subeffusis; acervula plurima (2—10), nigra, amphigena, subepidermica, dein ostiolato-erumpentia, minuta (0,2—0,4 mm. diam.), rotundata vel elliptica; conidiophoris dense parallelisque stipatis, erectis, simplicibus vel obsolete ramosis, 60—100 μ. longis, 0,6—1 μ. latis, delicatis, hyalinis, continuis; conidiis a conidiophoris parce distinctis, filiformibus, aciculatis, plus vel minus curvatis, hyalinis, continuis.

Experimental Actinomycosis[1]

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(1.2.1951)

Up to the present time, a considerable amount of work has been done in attempting to establish actinomycosis in experimental animals using known strains of the etiological agent, *Actinomyces bovis*. Certain workers such as ROSEBURY *et al.* (4) have shown that a filamentous anaerobic organism identified as *A. bovis* can be isolated from the apparently normal human mouth. These organisms have been used in the attempt to establish actinomycosis in experimental animals with varied but largely negative results.

It was our purpose first of all to isolate a filamentous organism from the normal mouth and identify it with a known strain obtained from an actual human case of actinomycosis. Secondly, we inoculated both strains into experimental animals in an attempt to initiate the disease and study the comparative results of its establishment by both strains. Serological studies were made to see if there was any relationship between immunological phenomena and establishment of the disease in any particular animal.

Source and Method of Isolation

Two organisms were used exclusively throughout these experiments. The first, a known strain of *Actinomyces bovis* (# 10049), was obtained from the American Type Culture Collection. This strain was isolated in 1946 from a human case of actinomycosis.

The second organism was isolated from the normal human mouth. Scrapings were taken with an inoculating wire from the hidden surfaces of apparently healthy teeth. These scrapings were streaked out on Bacto-brain heart infusion — 1 % glucose agar plates and incubated anaerobically at 37° C. After incubation for four to six days, the plates were removed and checked for colonies resembling *A. bovis*. Three principal methods described by ROSEBURY *et al.* (4) were employed in detecting these organisms. First the colonies were examined under the low and high power lens of the light microscope for evidence of mycelium extending from the periphery of the colony. Also roughness of the colony was taken into consideration. Second, a wet mount was made of a portion of a suspected colony and examined under the darkfield microscope for typical branching and diphtheroid forms. Third, a Gram stain was made in order to determine the staining reaction and to further confirm the presence of branching and diphtheroid forms.

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[2] A part of this investigation was used in partial fulfillment of the requirements for the degree of Master of Science.

A Comparison between the Filamentous Organism Isolated from the Normal Human Mouth and a Known Strain of Actinomyces bovis.

The characteristics of the two organisms were compared in a number of different ways: (1) morphology, (2) cultural requirements, (3) antigenic relationships, (4) degree of pathogenicity in experimental animals.

I. *Morphology*

Both the known strain of *A. bovis* and the filamentous organism showed rough, "heaped up" colonies with peripheral mycelium when grown on brain-heart infusion 1 % glucose agar plates. The only difference was that the known strain of *A. bovis* was more adherent to the media and its colonies were more difficult to emulsify. Gram stains of both organisms showed no difference between them. Both gave evidence of some branching but diphtheroid forms were predominant.

Some differences were noted between the two organisms in their respective growth in brain-heart infusion glucose broth. *A. bovis* showed consistent growth of white crumb-like colonies which remained at the bottom of the tube. The filamentous organism from the teeth grew as a wooly mass at the bottom of the tube and had a tendency to form a lattice growth on the side of the tube. This same type of growth was described by Slack (5) in connection with a strain of *A. bovis* isolated from the tonsillar crypts.

In thioglycollate fluid medium, both organisms again showed separate growth characteristics. *A. bovis* (known strain) grew as compact white granular colonies with a rough appearance. The mouth organism varied in this medium from diffuse growth to a white crumb-like growth surrounded by a wooly mass. It never showed the compact colony type of the known *A. bovis*. Conversely, *A. bovis* never grew diffusely in thioglycollate medium. It is interesting to note that Thompson (6), differentiating the bovine strain from the human strain of *Actinomyces*, attributes diffuse growth in thioglycollate media exclusively to the bovine strain. However it is also noteworthy that the filamentous organism isolated from the human mouth did show diffuse rather than compact colony growth in thioglycollate medium. Accordingly there may be some difference in the appearance of *A. bovis* in this particular medium as to whether its source in the human body is pathological or nonpathological. Gram stains of the type culture collection strain of *A. bovis* showed longer filaments whereas the other strain showed a shorter more branching mycelium. After continuous subculturing in thioglycollate medium, both organisms broke down into diphtheroid forms to the point where they were indistinguishable.

II. *Cultural Requirements*

The same conditions for culturing were employed in regard to both organisms. They both grew luxuriantly in Bacto brain-heart infusion 1 % glucose agar and in brain-heart broth as well as in Bacto thioglycollate fluid medium. The best growth was obtained at about pH 7.4. Glucose in a 1—2 % concentration in the media seemed to increase the amount of growth in both cases. Neither organism grew under aerobic conditions and a reduced oxygen tension and increased carbon dioxide tension were necessary for good growth.

Both organisms slowly fermented glucose, lactose, sucrose, mannitol and maltose with the production of acid but no gas.

III. *Antigenic Relationship*

A further indication of the close relationship of the filamentous mouth organism to *A. bovis* was demonstrated by its antigenic structure as compared with the known strain. Results of cross absorbtion experiments show only a slight difference in the antigenic structure of these two organisms (Table I). Actually it appears as though the known *A. bovis* (E strain) has more antigens. After absorbtion with the heterologous antigen from the mouth (M strain), there was only a slight reaction with its homologous antigen. However, more significant results were obtained when the serum of the filamentous organism (M strain), absorbed with the E strain, was tested with its homologous antigen. No reactions occurred in this experiment. A possible explanation for these results is that the known strain of *A. bovis* contained all the antigens present in the filamentous organism. The M strain had no antigenic specificity in addition to its relationship to *A. bovis,* otherwise a reaction would have occurred with its homologous antigen. From these results, it appears that the filamentous organism isolated from the normal human mouth possesses the same antigens as a known pathogenic strain of *A. bovis* isolated from a human case of actinomycosis. It is therefore strongly suggestive that the filamentous organism is *A. bovis.*

TABLE I. *Cross-absorption tests to determine antigenic relationships*

	M antigen	E antigen
M antiserum absorbed with E antigen . . .	negative	negative
E antiserum absorbed with M antigen . . .	negative	+ 1:40 ± 1:80 — 1:160
M antiserum unabsorbed	+ 1:5,120 ± 1:10,240 — 1:20,480	+ 1:2,560 ± 1:5,120 — 1:10,240
E antiserum unabsorbed	+ 1:2,560 ± 1:5,120 — 1:10,240	+ 1:10,240 ± 1:20,480 — 1:40,960

IV. *Degree of Pathogenicity in Experimental Animals*

No definite statement can be made about the comparative pathogenicity of the filamentous organism and the known strain of *A. bovis* as tested by animal inoculations. It may be of some significance that both organisms showed similar lesions along the marginal ear vein. However, no lesions positive by culture were produced by inoculation of the E strain either intraperitoneally or intravenously into experimental animals. The only conclusive evidence in comparing these two organisms in this respect was demonstrated by the fact that neither organism was very pathogenic in experimental animals.

On the basis of morphology, cultural requirements, fermentation reactions and antigenic relationships, there is an indication of a considerable similarity between these two organisms. In view of these facts, the organism isolated from the mouth can be identified as *A. bovis.*

Sensitization and Production of the Disease in Experimental Animals

Both the known strain of *A. bovis* and the filamentous organism isolated from the mouth were grown either in thioglycollate fluid medium, brain-heart infusion broth or veal infusion broth for inoculation into experimental animals.

The animals used for inoculations were guinea pigs and New Zealand white rabbits. The rabbits were all inoculated intravenously into the marginal ear vein. The guinea pigs were all inoculated intraperitoneally.

The amount of heavy suspension of viable organisms inoculated at any one time varied from 3ml to 7ml.

Since sensitization may play a role in the pathogenesis of actinomycosis, the schedule of inoculations was varied for different animals to see if this had an effect in the production of the experimental disease. Some animals were given one large dose and then after a considerable length of time they were autopsied. Other animals were inoculated at monthly intervals over a period of five months. Still others were given weekly inoculations and one animal was inoculated daily.

All animals were autopsied some time after inoculations were suspended. The autopsies were performed to detect any actinomycotic lesions within the animal body. If an actinomycotic lesion appeared on the outside surface of the body or if any suspicious lesions were found anywhere inside the animal body, direct smears and cultures were made from these lesions. If any typical actinomycotic organisms were detected in these media, an attempt was made to isolate the organisms in pure culture and to identify them as the organisms originally inoculated.

Results of Animal Inoculations

The results of animal inoculations in the attempt to establish actinomycosis were largely negative (Table 2). None of the animals died of the disease.

A total of eight rabbits were injected intravenously. Six of these rabbits were injected with the filamentous organism isolated from the mouth (M strain) The other two animals were inoculated with the known strain of *Actinomyces bovis* (E strain). All except two rabbits showed subcutaneous lesions along the marginal ear vein after the initial inoculation. They persisted for a relatively short time and then sloughed off and dissappeared. No additional lesions appeared upon further inoculation into either of the two marginal ear veins. No sulfur granules were ever noted in connection with these lesions. Gram staining of the exudate showed some branching and diphtheroid forms. In three cases, the M strain originally inoculated was reisolated from a lesion along the marginal ear vein.

Only one rabbit, RA (Table 2), showed any symptoms characteristic of actinomycosis. This animal was killed after six months of weekly inoculations. Autopsy showed a nodular lesion on the dorsal peritoneal lining. The lesion contained caseous material which gave evidence of branching organisms, but no club formation was seen upon gram staining. After reisolation, this organism was identified as the M strain originally isolated from the animal. The titer at the time of autopsy was positive to a serum dilution of 1:10,240.

Since agglutination reactions were not yet developed at the time of initial inoculation of rabbits designated as RA, RB, RC, and R2 (Table 2), no test was run to determine a titer prior to the start of inoculations.

Rabbits R1 and R2 inoculated with the E strain also showed no lesions upon autopsy. Periodic agglutination tests were run against the serum of rabbit R1. The results of these tests are tabulated in Table 3.

Five guinea pigs were inoculated intraperitoneally with both organisms. One of the three guinea pigs inoculated monthly with 3.5 ml of the M strain over a period 221 days showed typical actinomycotic lesions. This animal (GI) developed three lesions in the peritoneal lining. One lesion was located in the connective tissue between the epidermis and the peritoneal lining on the left midventral abdominal region overlying the stomach. This lesion contained the organism in the form of sulfur granules showing club formation.

Another lesion about three centimeters in diameter was located in the inner peritoneal lining of the left ventral abdominal region adjacent to the stomach. The left lobe of the liver was adhered to this lesion. Nevertheless, neither the liver nor the stomach appeared to be infected. The lesion itself was walled off and contained much caseous exudate but no sulfur granules. A Gram stain of this exudate showed diphtheroid forms without clubs.

The third lesion occurred on the surface of the peritoneal lining in the area of inoculation. Its position was midventral in the lower abdominal region. The lesion itself was approximately two centimeters in diameter. Caseous material was found inside the lesion. Gram stains of this material showed Gram positive diphtheroid forms without branching or club formation. The strain inoculated was reisolated and identified from this lesion as well as from the second lesion described above.

Although the peritoneal lining of this animal had typical actinomycotic lesions in two different places, there was no evidence of infection of the viscera.

During the period of injections, both guinea pigs GI and GII showed extensive subcutaneous lesions in the abdominal region. These lesions spread underneath the skin breaking through the surface at various places. However there was no evidence of penetration of the peritoneal lining. A large amount of caseous material was present in the lesions although no sulfur granules were found. Reisolation of the filamentous organism was difficult because of a great predominance of anaerobic streptococci. After a thorough cleaning, the lesions healed completely in the case of both guinea pigs.

Guinea pig GA (Table 2) showed numerous lesions on the peritoneal lining of the lower abdominal region. Gram stains of the exudate from the lesions showed branching organisms with club formation. The original E strain could not be reisolated from the lesion because of an overgrowth of anaerobic streptococci.

The only agglutination tests made on guinea pigs were immediately before autopsy.

Discussion of Animal Pathogenicity

The most successful results from intravenous inoculations of rabbits with *Actinomyces bovis* were described by SLACK (5). He was able to produce progressive malignant actinomycosis in four out of five rabbits. The attempt to duplicate Slack's findings by using the M (mouth) and E (known) strain organisms for intravenous inoculations was unsuccessful. It is interesting to note that rabbit RA, described previously, developed a lesion in the peritoneal lining after intravenous inoculations. Since streptococci were present in this lesion, it cannot be definitely stated that the causative agent of this infection was solely the M strain organism. However, it is of significance that this organism was reisolated from the lesion and identified as the same organism originally inoculated. This indicates that the M strain was probably the initial causative agent of the lesion. There is a possibility here that the streptococci may have been a secondary invader and may have enhanced the development of the lesion. NAESLUND (3) was able to show and EMMONS (1) suggested that other organisms play a role in the initiation and development of actinomycotic lesions.

Since no extensive actinomycosis occurred in any animal, it was impossible to draw any correlation between the titer in the animal blood serum and the progressiveness of the disease. However, from the data given on rabbit RA (Table 2), it can be seen that a significantly higher titer does accompany the incidence of lesions in the animal. This was also true of the guinea pig GI (Table 2). Even though these were the only two animals which showed lesions upon autopsy, these results indicate that a large amount of antibodies do not serve as a protection to an actinomycotic infection. It is apparent that inoculations continued over a long period of time seem to be more effective than huge doses given for a short time. The establishment of lesions seems to depend upon the degree of sensitization of the animal. This viewpoint was advocated earlier by MATHIESON *et al.* (2). These workers were able to produce lesions in guinea pigs only after repeated inoculations.

The titer in rabbits inoculated with either strain remained relatively high over a considerable length of time after the terminations of inoculations (Table 2). This was especially true of rabbits RC and RB. One rabbit, Rl, showed a very gradual decrease in titer over a period of 80 days following the last injection (Table 3). This may indicate that there is a constant production of antibodies stimulated by living and reproducing actinomycotic organisms in the body tissues of the animal.

Of all the animals inoculated, only one guinea pig, GI, showed signs of a possible progressive actinomycotic infection. During the course of inoculations, subcutaneous suppurating lesions appeared in the area of inoculation. It cannot be stated definitely that this lesion was caused by the M strain because of the large number of streptococci in this lesion. The general appearance of the lesion fits the description of the actinomycotic lesion. The fact that the infection spread out between the skin and the peritoneal lining without affecting this lining is in full accord with observations made by earlier investigators. NAESLUND (3) found that an actinomycotic process can spread out extensively under the skin and mucous membranes themselves. The subcutaneous lesion in the guinea pig healed up completely upon drainage.

This particular guinea pig was the only animal which showed loss in weight during the period of inoculations. The loss in weight was rather abrubt but the animal did not die after a considerable period of time after this condition was first noted. According to SLACK (5), loss of weight was reported as an indication of progressive actinomycosis in rabbits.

The post mortem of this guinea pig revealed extensive nodular lesions in the peritoneal lining. It is of interest that the left lobe of the liver was adherent to this lesion but there was no gross evidence of infection in the liver itself. The prevention of further spread of the infection could have been due to the fact that the lesion was walled off so completely.

Most of the exudate obtained from the lesions in animals during these experiments did not show sulfur granules. However, the exudate taken from lesions on the peritoneal lining of two guinea pigs, GI and GA did show sulfur granules. Sulfur granules seem to be present only when there are extensive lesions in the experimental animal and both sulfur granules and lesions were seen only in animals with a higher titer of circulating antibodies. Club formation was evident in the granules taken from the lesion in the guinea pig GA. At times Gram stains of the supperative material showed diphtheroid forms rather than filamentous forms. Branching organisms also appeared in the exudate which did not contain granules. This indicates that the organism may be present in these lesions in various forms.

TABLE II. *Results of animal inoculations.*

Strain	Animal	Period of Injections	Average Dose Injected ml.	Total Amount Injected ml. (conc. inoc)[1]	Length of time After Last Inj. to Autopsy (days)	Titer Time of Antopsy (Dil. of Antiserum)	Lesions at Autopsy	Reisolation of organism from Lesion
M	RA	180 days weekly	3.5	88.7	3	1:10,240	peritoneal lining	+
M	RB	31 days weekly	4.0	19.0	164	1:2,560	none	—
M	RC	1 day	10.0	10.0	175	1:160	none	—
M	RD	101 days monthly	3.5	17.5	3	1:5,120	none	—
M	RE	77 days monthly	4.0	14.0	3	1:640	none	—
M	RF	6 days daily	3.0	18.0	3	1:5,120	none	—
M	GI	221 days monthly	3.0	30.0	8	1:10,240	peritoneal lining	+
M	GII	130 days monthly	4.0	26.8	1	1:1,280	none	—
M	GIII	56 days weekly	3.5	25.0	173	1:640	none	—
E	R1	102 days monthly	3.5	25.5	93	1:1,280	none	—
E	R2	30 days weekly	4.0	20.0	162	1:160	none	—
E	GA	61 days weekly	3.5	30.0	41	......	peritoneal lining	—
E	GB	1 day	7.0	7.0	101	negative	none	—

M filamentous org. isolated from the mouth used as antigen. E known strain *A. bovis* used as antigen. R rabbit; G guinea pig; + org. was reisolated from the lesion; — org. was not reisolated from the lesion.

TABLE III. *Periodic titer of rabbit serum*

Aggl. Test	Strain	Animal	No. Injections at Time of Test	Total Dosage of Antigen at Time of Test (ml)	Total Dosage Time Interval Since Last Test in Days	Titer Dilution of Antiserum
1st	M	RD	2	7.0	20	+ 1:1280 ± 1:2560 — 1:5120
2nd	M	RD	1	10.5	60	+ 1:640 ± 1:1280 — 1:2560
3rd	M	RD	0	10.5	10	+ 1:640 ± 1:1280 — 1:2560
4th	M	RD	2	17.5	8	— 1:5120 ± 1:10240 — 1:20480

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[1] The density of the inoculum was greater than the No. 10 tube of the McFarland nephelometer. The organisms were grown in tubes containing 10 ml of broth. One ml of the inoculum was represented by the growth from 10 ml of broth.

TABLE III. *Continued*

Aggl. Test	Strain	Animal	No. Injections at Time of Test	Total Dosage of Antigen at Time of Test (ml)	Total Dosage Time Interval Since Last Test in Days	Titer Dilution of Antiserum
1st	M	RE	1	3.5	8	+ 1:1280 ± 1:2560 — 1:5120
2nd	M	RE	1	7.0	68	+ 1:160 ± 1:320 — 1:640
3rd	M	RE	2	14.0	8	+ 1:640 ± 1:1280 — 1:2560
1st	M	RF	2	6.0	2	+ 1:2560 ± 1:5120 — 1:10240
2nd	M	RF	2	12.0	2	+ 1:2560 ± 1:5120 — 1:10240
3rd	M	RF	2	18.0	2	+ 1:5120 ± 1:10240 — 1:20480
1st	E	R1	2	10.0	13	+ 1:640 ± 1:1280 — 1:2560
2nd	E	R1	3	20.5	66	+ 1:5120 ± 1:10240 — 1:20480
3rd	E	R1	1	25.5	15	+ 1:20480 [1] ± 1:40960 — 1:81920
4th	E	R1	0	25.5	54	+ 1:2560 ± 1:5120 — 1:10240
5th	E	R1	0	25.5	11	+ 1:1280 ± 1:2560 — 1:5120

Controls run on the serum of these rabbits prior to the initial injection were negative. The period of injections can be seen in Figure 1.

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[1] Three tests were run to verify this titer since it was unusually high.

Summary and Conclusions

1. A filamentous organism (M strain) was isolated from the scrapings of the teeth from the normal human mouth. This organism was identified as *Actinomyces bovis* by comparing it with a known organism (E strain) on the basis of morphology, cultural requirements and antigenic relationships.

2. Colony characteristics of both organisms on solid media were very similar. Gram stains as a rule showed no differences in the morphology of the organisms especially after repeated subculturing. Some differences in colony appearances were noted in Difco thioglycollate fluid and brain-heart infusion broth media.

3. The cultural requirements of both organisms were identical. Both grew equally well on the same media if the glucose content was increased to 1 or 2 %. A reduced oxygen and increased carbon dioxide tension was necessary for growth. Optimum growth was obtained at pH 7.2—7.6. Both strains slowly fermented glucose, lactose, sucrose, mannitol and maltose with the production of acid but no gas.

4. There was a distinct antigenic similarity between the two organisms. No antigenic specificity was observed in the case of the M antigen in its relationship to the E antigen as determined by cross absorption tests.

5. Since both the M and E strains were relatively non-pathogenic, no conclusive comparison could be drawn between these two organisms as to their relative pathogenicity in experimental animals.

6. In most instances, inoculations by intravenous and intraperitoneal routes in rabbits and guinea pigs respectively gave negative results in an attempt to establish actinomycosis in these animals. However, one rabbit and one guinea pig showed lesions in the peritoneal lining after autopsy. The guinea pig developed subcutaneous lesions during the period of inoculations and showed some signs of progressive actinomycosis. Both animals were inoculated repeatedly with the M strain over a long period of time.

7. There is evidence that a high antibody content in the blood stream of experimental animals does not protect the animal against infection. On the contrary, both of the infected animals were among those showing the highest titer of antibodies by agglutination tests. This may indicate that the sensitization is an essential factor in the establishment of the disease.

Inhaltsübersicht-Experimentelle Actinomykose

1. Ein fadenartiger Strahlenpilz (M Stamm) wurde von dem Ansatz der Zähne im normalen menschlichen Munde empfangen. Dieser Strahlenpilz wurde identifiziert als *Actinomyces bovis* durch Vergleichung mit einer bekannten Art dieses Organismus (E Stamm). Diese Vergleichungen wurden auf Grund von Morphologie, Kulturnotwendigkeiten und antigene Verwandschaften gemacht.

2. Morphologie der Colonien beider Organismen am festen Nährboden war ziemlich dieselbe. Gramfarbung zeigten gewöhnlich keinen Unterschied zwischen beiden Organismen überhaubt nach fortwährender Verpflanzung. Etliche Verschiedenheiten in den Ansichten der Colonien machten sich bemerkbar in „Difco thioglycollate" und „Difco brain-heart infusion" Flüssigkeitsnährböden.

3. Die Kulturnotwendigkeiten beider Organismen waren überhaubt gleich.

4. Eine gewisse antigene Vergleichung war ganz bestimmt bemerkbar in Bezug auf beide Stämme. Keine antigene Specificität des M Stammes wurde

bemerkt in dessen Bezug auf den E Stamm als bestimmt durch Kreuzabsorbtionsreaktionen.

5. Da die Pathogenese beider Organismen in den Versuchstieren nicht sehr bemerkbar war, konnte keine Vergleichung in dieser Hinsicht angestellt werden.

6. In den meisten Fällen waren die Resultate für die Einsetzung der Actinomykose negativ nachdem Injektionen mit den zwei Organismen in die Venen von Kaninchen und in die Bauchhölen von Meerschweinen gemacht worden waren. Doch in Kaninchen und in Meerschweinen zeigten Läsionen in den Peritonäalüberzug nachdem man diese Tiere aufgeschnitten hatte. Das Meerschweinchen zeigte auch Läsionen gleich unter der Haut des Unterleibes während Injektionen in dieser Umgebung gemacht wurden. Dieses Tier gab gewisse Beweise dafür dass eine fortschreitende Actinomykose hier der Fall war. Beide Tiere bekammen Injectionen mit dem M Stamm während langer Zeit.

7. Es gibt auch Beweis dafür dass ein höher Antikörpersgehalt im Blut der Versuchstiere das Tier nicht gegen Infektion mit *Actinomyces bovis* schützt. Im Gegenteil, beide angesteckten Tiere zeigten den höchsten Titer von Antikörper. Das kann daraufhinweisen dass Senzitation als ein notwendiger Factor für die Einsetzung der Aktinomykose angesehen werden kann.

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Notas sôbre a denominação genérica e especifica do agente da blastomicose Sul-Americana. Comentários sôbre uma questão de nomenclatura botânica médica.

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(1.1.1951)

Desde 1.930, a grande maioria dos micologistas utiliza para designar o agente da blastomicose sul-americana a denominação *Paracoccidioides brasiliensis* (Splendore 1912) Almeida 1930. Com esta denominação, Almeida (1930) revalidou e redescreveu a espécie *brasiliensis* de Splendore (1912), dando ao referido germe seu justo lugar em um novo gênero-*Paracoccidioides*. Durante muito tempo foi a nossa blastomicose confundida do ponto de vista clínico e etiológico com a granulomatose coccidióidica, observada pela primeira vez na Argentina por Posadas e Wernicke e depois por Rixford e Gilchrist nos Estados Unidos. Coube a Floriano de Almeida o mérito de ter mostrado as diferenças entre as duas entidades, individualizando de modo perfeito o agente da nossa blastomicose. A este respeito afirmam Redaelli e Ciferri (1937) à página 599 de sua monografia: ,,Prendiamo dalle opere di Almeida i dati che si riferiscono alla epidemiologia, alla clinica ed alla anatomia patologica del granuloma coccidioide, perché é dai lavori di questo Autore che la forma morbosa ha acquistato oggi una individualitá ben definita''.

Os trabalhos de Almeida foram realizados comparando numeroso material norte americano que lhe foi enviado por Evans, Weidmann, Moore e muitos outros micologistas norte-americanos.

Universalmente foram aceitos os resultados de tais pesquisas, sendo que em 1937, dois eminentes micologistas italianos — Redaelli e Ciferri publicaram interessante monografia sôbre o assunto, aceitando sem restrições os trabalhos de Almeida e colocando o gênero *Paracoccidioides* em nova família — *Paracoccidioidaceae,* afastando-o, portanto, mais ainda do gênero *Coccidioides.*

Dodge (1935) em seu livro — ,,Medical Mycology'', aceita o gênero *Paracoccidioides* com a espécie *brasiliensis.*

Gougerot (1936) na ,,Nouvelle Pratique Dermatogique'' estabeleceu as diferenças entre *Coccidioides immitis* e *Paracoccidioides brasiliensis*, aceitando igualmente esta denominação para o agente da nossa blastomicose. Nos Estados Unidos, Evans, Moore, Weidman, Jordan e muitos outros manifestaram-se, depois de estudos cuidadosos, favoraveis aos resultados das pesquisas empreendidas por Almeida.

Em 1936, Joran e Weidman, tendo em vista tais estudos, propuzeram a denominação de Molestia de Almeida (Almeida's Disease) à granulomatose paracoccidióidica.

Redaelli e Ciferri (1937), assim como Moore (1938) denominaram tal entidade — ,,Moléstia de Lutz-Splendore-Almeida''. Brumpt, (1936) em seu ,,Précis de Parasitologie'' e Nannizi (1934) em seu ,,Repertorio Sistematico dei Miceti dell'Uomo e degli Animali" aceitam igualmente o gênero *Paracoccidioides* e a espécie *brasiliensis*.

NEGRONI (1937) e NIÑO (1939), na Argentina, assim como numerosos outros micologistas aceitam tal designação genérica e específica.

No grande tratado alemão ,,Handbuch der Haut und Geschlechtskrankheiten", dirigido por JADASSOHN, ROCHA LIMA (1932) escreveu o capítulo das ,,Blastomicoses exóticas" no qual o novo gênero *Paracoccidioides* foi estudado e considerado como o causador da forma brasileira. AMADEU FIALHO (1946) tambem aceita a espécie *brasiliense* de SPLENDORE, fazendo estudo cuidadoso a êste respeito.

Há alguns anos e contrariando tudo o que acima ficou exposto, FONSECA FILHO (1939), aceitando as diferenças entre *Coccidioides immitis* e *Paracoccidioides brasiliensis*, pretendeu invalidar a espécie criada em 1912 por SPLENDORE, para dar ao germe da nossa blastomicose um novo gênero que levaria a espécie *histosporocellularis*, descrita sete anos mais tarde por HABERFELD. Vamos mostrar, citando trechos de trabalhos de diferentes AA., e portanto com um mínimo de cunho pessoal, que a espécie descrita em 1912 por SPLENDORE é perfeitamente válida e de acôrdo com a lei de prioridade deve ser mantida, assim como o gênero *Paracoccidioides* ALMEIDA 1930.

Quem conhece a bibliografia sôbre tal assunto, verifica que em tudo isto há uma sucessão de fatos mal observados, fatos êstes que vêm trazer ao espírito dos que não são especialistas, grande confusão, e confusão em assunto de relevância, pois trata-se da justa denominação a uma infecção micótica que representa algo de importante em nossa nosologia, como afirmou SIMÕES BARBOSA (1940).

Já em 1940, este micologista pernambucano, numa análise imparcial, historiando o estudo da nossa blastomicose, mostrou de modo irrefutável que a unica denominação válida para o agente da moléstia de LUTZ-SPLENDORE-ALMEIDA era a de *Paracoccidioides brasiliensis* e que *Lutziomyces histosporocellularis*, por supérfluo, deveria passar para a sinonímia de *Paracoccidioides brasiliensis* ou então ser regeitado.

Em taxonomia, diz FONSECA FILHO (1943) à página 5 de sua ,,Parasitologia Médica", referindo-se à lei de prioridade que consiste em considerar como unico válido o nome mais antigo aplicado a cada ser vivo: ,,*o nome mais antigo prevalece, ainda que tenha sido aplicado apenas a um sexo, a uma fase do ciclo evolutivo ou a uma simples porcão de organismo da espécie em questão*".

Desejando proporcionar dados históricos de grande significação aos que desconhecem a literatura antiga sôbre o assunto e mais ainda, em respeito à verdade científica, resolvemos trazer ao conhecimento dos colegas êstes fatos, mostrando com um mínimo de cunho pessoal, que a unica denominação válida e que está de acôrdo com as regras de nomenclatura botânica para designar o agente da nossa blastomicose é *Paracoccidioides brasiliensis* (SPLENDORE 1912) ALMEIDA 1930.

Em 1908, LUTZ publicou os dois primeiros casos de granulomatose paracoccidióidica no Brasil, dando completa descrição do parasito. Já nesta época LUTZ, que teve oportunidade de examinar o doente de POSADAS, aifrmava as diferenças entre os dois parasitos, dizendo textualmente: ,,nos meus casos nunca vi esporulação endógena e me custa um pouco acreditar na sua realidade."

LUTZ (1908) em um de seus casos obteve, em cultura, um cogumelo possivelmente de contaminação, quando afirmou: ,,Do tecido da glandula linfática e salivar obtive sem dificuldade culturas puras com os caracteres do *Oidium lactis* e do fungo do sapinho; as experiências feitas com êste fungo deram um resultado positivo numa cobaia que, depois de seis mezes ofereceu muitas glandulas linfáticas hipertrofiadas contendo o fungo em forma de pseudococcídios. O cogumelo cresce em quase todos os meios nutritivos com bastante rapidez".

No seu trabalho, LUTZ afirma: ,,O Dr. A. SPLENDORE tambem fez ultimamente duas observações desta afecção e das quaes uma com localização cutânea e mucosa".

Neste mesmo ano, CARINI (1908) observa um caso de granulomatose paracoccidióidica com localização primitiva na mucosa bucal (palato) sem enfartamento ganglionar.

Em 1909, SPLENDORE observa novo caso de blastomicose generalizada cujos caractéres clínicos eram muito semelhantes a outro por ele anteriormente comunicado à Sociedade Científica de São Paulo.

Ainda neste ano, LINDENBERG em seu trabalho sôbre as ,,Dermatomicoses brasileiras", estuda do ponto de vista clínico a granulomatose paracoccidióidica. Em 1910, SPLENDORE, num relatório apresentado ao IV° Congresso Médico Latino Americano, estudando as blastomicoses americanas e referindo-se aos casos de blastomicose observadas até esta época, afirma: ,,estes casos, todos mais ou menos semelhantes entre si, são caracterizados pela localização primitiva da lesão na mucosa da boca". E prosseguindo, à página 8 do seu trabalho, diz: ,,Em dois casos, o início foi propriamente na commissura labial e num terceiro na gengiva. Os pacientes se queixavam com frequencia de dificuldade na deglutição; alguns tinham enorme secreção salivar; quasi todos acusavam extraordinário cansaço geral sempre crescente. Alguns morreram em seguida a extrema caquexia; outros estão vivos ainda, mas não apresentam melhoras notaveis. Nenhum remedio local ou geral deu até agora bom resultado". Citando o trabalho de LUTZ, diz SPLENDORE: ,,Quanto aos meus casos que são três, devo dizer que num, cujo exame foi limitado ao material colhido pela raspagem da lesão, *obtive cultura de um cogumelo completamente semelhante ao que foi isolado pelo* Dr. LUTZ, *apresentando as colônias, especialmente em agar-glicerinado, o aspecto caracteristico filamentoso que por alguns observadores foi comparado a pele de ratinhos brancos.*" A página 11, diz: ,,*Num segundo caso encontrei, nos tecidos patológicos, diversos corpusculos parasitarios, analogos aos que notou o* Dr. LUTZ; mas vi em diversos preparados formas indubitavelmente gemeadas, mesmo sendo o material imediatamente fixado em formalina a 10 %; eu tambem nunca encontrei esporulações endogenas".

RABELO (1912), em seu trabalho — ,,Contribuição ao estudo das dermatomicoses", fez estudo sobretudo clínico de três casos desta infecção. Em 1911 SPLENDORE volta a esmiuçar o assunto num trabalho sôbre ,,Bouba-Blastomicose — Leishmaniose" e afirma textualmente:" nos de natureza micótica, o inìcio do processo pathologico foi sempre na boca: uma vez na base da lingua, outra nos labios ou na gengiva, propagando-se as mais das vezes à aboboda palatina, à pharynge, à larynge, aos bronchios, aos pulmões e a mais órgãos internos".

E prosseguindo: ,,Em alguns casos notavam-se erozões ou ulcerações pouco profundas, situadas com especialidade sobre o véo palatino". Referindo-se ao agente etiológico encontrado nas lesões, diz SPLENDORE: ,,estes corpos foram encontrados com relativa frequencia, no exame a fresco do material retirado directamente das lesões, independentemente de qualquer reagente ou coloração e mais facilmente ainda, com auxilio de uma fraca solução de soda ou potassa; algumas vezes, porém, não foi muito facil a sua constatação. Eles se apresentam, de ordinário, sob dorma redonda ou redondoide, com duplo contorno, tendo, em alguns casos, aspecto de pseudo-coccideos intra ou extracellulares".

Diz, a seguir: ,,. . . os referidos corpusculos differem dos descriptos pelos observadores argentinos pelo fato de frequentemente se apresentarem tambem gemmeados e não apresentarem a esporulação endogena n'aqueles descripta". Em 1911, PEREIRA e GASPAR VIANA observaram um caso de blastomicose generalizada; MONTENEGRO (1911) relata uma observação de forma linfatico-tegumentar e JOSÉ FRANCO DE CASTRO CARNALHO (1911) defende no Rio de Janeiro sua tese de doutoramento sôbre uma forma rara de blastomicose (forma mucosa, simulando sifiloma hipertrófico difuso do labio).

Em 1912, SPLENDORE publica no ,,Bulletin de la Societé de Pathologie Exotique" (tome V—nº 5) um trabalho — ,,Zymonematosi com localizzacione nella cavitá della bocca, observata in Brasile" e referindo-se ao cogumelo agente das lesões diz: ,,L'aggruppamento dei parassiti é, non di rado, carateristico, mostrandosi formato di una forma maggiore circondata da numerosi altre minori, derivanti, indubiamente, da gemmazione della precedente".

Ainda neste ano, o mesmo Autor — no ,,Giornale di Biologia, Medicina e Chirurgia (ano VII — nº 15) publica novo trabalho sobre ,,Blastomicose, Sporotricosi e rapporti com processi affini".

Diz o referido Autor à pág. 6: ,,Existe per, in Brasile, un tipo clinico di blastomicose si da me particolarmente illustrado che é caratteristico per la localizzazione delle sue lesioni principali nella cavitá della bocca. La sua constatazione fu fatta per la prima volta da A. LUTZ". E referindo-se ao parasito diz: ,,Detti corpi possono essere facilmente messi in evidenza nei preparati microscopici indipendentemente da reativi o colorazione".

O trabalho fundamental de SPLENDORE foi publicado na Italia, em 1912, num volume comemorativo do 25° aniversário de ensino do Prof. ANGELO CELLI.

Neste magnífico trabalho, SPLENDORE estuda quatro casos de granulomatose paracoccidióidica e descreve com precisão os caractéres clínicos dessa infeçcão micótica. A página 422 diz: ,,Trattasi di una malattia micotica speciale dominante in Brasile la quale peró essere classificata fra le cosi dette blastomicosi generalizzate descritte principalmente nell'America del Nort, mas, per la localizzazione specifica delle sue lesione nella cavitá della bocca non trova, fino al ora, riscontro nella patologia di alti paesi". A descrição clínica que SPLENDORE fez da granulomatose paracoccidióidica é perfeita e aqueles que estudam com cuidado esta molestia verificam que o bacteriologista italiano, em todos os seus trabalhos é preciso quando analisa a fisionomia clínica dos casos que observou.

REDAELLI e CIFFERRI (1937) afirmam à página 600 do seu trabalho: ,,Successivamente SPLENDORE studiò, durante vario tempo, diversi casi, ed in base allo studio minuzioso dell parassita descrisse lo *Zymonema brasiliensis*". FONSECA FILHO afirma, no entanto, em seus trabalhos (1939—1943) que SPLENDORE não observou na realidade casos de granulomatose paracoccidióidica. Diante do que anteriormente relatamos, não há duvida alguma de que SPLENDORE realmente observou casos de blastomicose sul-americana.

O que lemos anteriormente é mais do que suficiente para não deixar qualquer duvida no espírito daqueles que querem julgar as coisas de modo imparcial.

Seria pouco lógico pretender filiar os casos de SPLENDORE a outras doenças micóticas, como por exemplo a doença de Gilchrist, excepcional no Brasil. Além do mais, o proprio SPLENDORE afirma que os seus casos são idênticos aos de LUTZ e os deste ultimo pesquizador aceita-os o Prof. O. DA FONSECA FILHO, como sendo de ,,blastomicose".

Assim à página 425 de seu trabalho, referindo-se ao seu primeiro caso, diz SPLENDORE: ,,In vista di tale reperto, non resta dubbio che il caso sia identico a quello del LUTZ". O proprio HABERFELD (1919) que decreveu a especie *histosporocellularis* sem ter conhecimento deste ultimo trabalho de

SPLENDORE, mas que apenas conhecia a primeira publicação deste Autor (1909) afirma à página 81, descrevendo o aspecto macro e microscópico das culturas do cogumelo: ,,podemos afirmar que a descrição do aspecto microscópico das culturas do germen que nos aqui interessa, foi feita por A. LUTZ e SPLENDORE, os primeiros observadores desta enfermidade no Brasil, de tal maneira que restam poucos fatos, dignos de especial interesse."

A análise cuidadosa e imparcial dos trabalhos de SPLENDORE nos conduz a uma unica conclusão: as suas observações clínicas são cuidadosas, e todas elas se referem a uma doença ,,que non trova riscontro nella letteratura medica di altri paesi".

É absurdo, portanto, se querer filiar os casos de SPLENDORE a uma outra etiologia. FONSECA FILHO (1943) afirma que SPLENDORE observou casos de doença de Gilchrist, mas à página 679 do seu livro (1943) descrevendo esta doença diz: ,,a raridade das lesões mucosas é uma das características da doença de GILCHRIST, permitindo distinguí-la da doença de LUTZ." Quanto à morfologia dos parasitos nas lesões, SPLENDORE descreveu-a com precisão de mestre quando diz: ,,L'aggrupamento dei parasiti é non de rado caratteristico, mostrandosi formato da una forma maggiore circondata da numerose altre minori derivanti, indubiamente, da gemmazione della precedente".

No seu primeiro caso, SPLENDORE observou ,,alcuni corpicciuoli di forma retonda a doppio contorno e contenuto granulare, ricordando talora la forma di pseudo-coccidi, ma mostrando non di rado, una gemmazione periferica a modo di torule, avente ciascun elemento dimensione varia, con diametro de 50 a 20 e piu u. In vista di tale reperto, non resta dubbio che il caso sia identica a quello del LUTZ".

No terceiro caso observado, SPLENDORE descreve formas catenuladas e o FONSECA FILHO (1939) afirma que tal aspecto não se encontra no parasito da nossa blastomicose. Ora, todos nós que cuidamos da parte micológica, sabemos como o *Paracoccidioides brasiliensis* pode simular brotamento e como cadeias de 4 a 5 e mesmo mais elementos são disposições artificiais muito comuns no parasito do granuloma paracoccidióidico. No que diz respeito às culturas, vejamos o que SPLENDORE conseguiu. A cultura do primeiro caso foi negativa.

No caso 2 (S.R., italiano), o doente não deixou fazer uma biopsia da boca, mas examinando o material (exame a fresco), diz SPLENDORE: ,.Um exame diretto a fresco mette in evidenza la presenza di rari corpicciuoli parasitari rotondi o rotondoidi a doppio contorno e contenuto granulare, i cui caratteri morfologici sono completamente rassomiglianti a quelli dei casi sopra ricordati".

Obteve deste caso cultivos típicos do *Paracoccidioides*, quando afirma à página 428: — ,,le colonie si presentavano dapprima rotonde, lisce, splendenti e poco sollevate, per ricoprirsi poi in seguito specialmente nei terreni glicerinati o glucosati, di una superficie vellutata, assumendo l'aspetto che fu paragonato alla pelle di topolino bianco". E à página 429 diz: ,,I caratteri colturali gel germe, d'altra parte, assolutamente indentici a quelli del microrganismo del caso di LUTZ, col quale vennero minuziosamente comparati, mettono la questione fuori discussione". Em relação às culturas de leveduras contaminantes obtidas por SPLENDORE no seu 3° e 4° casos, isto nada tem de extraordinario, pois o *Paracoccidioides*, como sabemos, é dificilmente cultivavel partindo-se de lesões abertas, e leveduras são frequentemente isoladas de lesões paracoccidióidicas. Aliás, o próprio LUTZ (1908) isolou leveduras de lesões blastomicóticas que descreveu.

Do que acabamos de expor, transcrevendo trechos de varios trabalhos de SPLENDORE, resalta claramente um fato: a espécie *brasiliensis* é válida e deve ser mantida de acôrdo com a lei da prioridade, pois diz. FONSECA FILHO à página 5 de sua Parasitologia Médica (1943) ,,o nome mais antigo prevalece, ainda que tenha sido aplicado apenas a um sexo, a uma fase do ciclo evolutivo ou a uma simples porção de organismo da espécie em questão". A unica objeção aos trabalhos de SPLENDORE que poderia ser feita era a de que ele não obteve cultura do parasito em todos os casos ou que no seu terceiro caso tenha trabalhado com uma levedura contaminante. Mas, mesmo assim não seria invalidada a denominação específica, pois claramente ficou provado que SPLENDORE, em todos os seus trabalhos descreve o parasito com a sua morfologia típica, tanto ao exame direto como em cortes, e fornece da nossa blastomicose minuciosa descrição clínica. Logo, não encontramos, em absoluto, base alguma para, invalidando a espécie de SPLENDORE, como quer FONSECA FILHO (1939) colocar o germe da nossa blastomicose em novo género.

A espécie criada por SPLENDORE não fére nenhum dispositivo das Regras Internacionais da Nomenclatura Botânica.

DODGE (1935), citando tais regras, diz:

,,Art. 63 — Um nome de um grupo taxonômico deve ser regeitado quando sua aplicação é incerta (nomen dubium).

,,Art. 64 — Um nome de um grupo toxonômico deve ser regeitado si os caractéres deste grupo forem derivados de dois ou mais elementos inteiramente discordantes, especialmente si aqueles elementos foram erroneamente admitidos para formar parte do mesmo individuo (nomina confusa). Ora, a espécie de SPLENDORE não poder ser considerada ,,Nomen dubium" ou ,,Nomen Confusum", como pretende o Prof. OLIMPIO DA FONSECA FILHO (1939). Em 1° lugar, sua significação não é incerta. Em 2° lugar, ela nunca se prestou a confusão ou erros.

Em 1930, ALMEIDA retomou, revalidou e redescreveu a espécie *brasiliense* de SPLENDORE, colocando-a em um novo gênero-*Paracoccidoides*. Já vimos anteriormente a aceitação universal que os trabalhos de ALMEIDA tiveram nos grandes centros micológicos.

Quer FONSECA FILHO (1939) invalidar a espécie *brasiliensis* para destruir o gênero *Paracoccidioides* de ALMEIDA.

É mais que supérfluo querer colocar o parasito da nossa blastomicose em novo gênero e muito menos tomar como tipo a espécie *histosporocellularis* de Haberfeld.

Vejamos o Artigo 16 das ,,Regras Internacionais de Nomenclatura Botânica:"

,,Art. 16 — Cada grupo com uma circunscrição, posição e categoria dadas, pode ter somente um nome válido, o mais antigo, com a condição de que esteja de acôrdo com as Regras de Nomenclatura.

Art. 60 — Um nome deve ser regeitado si for ilegitimo.

Um nome é ilegitimo nos seguintes casos:

1°) — Si for supérfluo quando publicado, isto é, si havia um nome válido para o grupo do qual foi aplicado, com sua circunscrição particular, posição e categoria".

Conclusão unica: deve ser regeitado por supérfluo o binômio ilegítimo *Lutziomyces histosporocellularis* FONSECA 1939. Si não se justifica criar novo gênero para o agente da nossa blastomicose, muito menos se deve tomar a espécie de Haberfeld como tipo. Quem lê a parte bacteriológica do trabalho de Haberfeld (1919) que desconhecia as ultimas publicações de SPLENDORE, verifica que aquêle Autor descreve o agente da nossa blastomicose como um cogumelo que ,,*se propaga unicamente por formação de endosporos*" e em culturas apresenta ,,celulas conidianas laterais" lembrando o *Microsporon Audouini* ou formas conidianas terminais semelhantes ao do cogumelo da esporotricose.

HABERFELD deu o nome de *Zymonema histosporocellularis*, ao parasito, esta última palavra querendo significar, diz o Autor em palavras grifadas: „*tratar-se de um germen do grupo dos Zymonemas que nos tecidos existe exclusivamente sob a forma de cellula e aqui se propaga unicamente por formação de endosporos*". Como se vê, a descrição é das mais infelizes e imprecisas.

Consequentemente, concluimos: Deve ser mantido o binômio *Paracoccidioides brasiliensis* (SPLENDORE 1912) ALMEIDA 1930 para designar o agente da blastomicose brasileira ou sul-americana.

Em 1941 e 1942 CONANT e HOWELL Jor. publicaram dois trabalhos, nos quais incluem o agente da nossa blastomicose no gênero *Blastomyces*.

Segundo esses AA. há muita semelhança entre os fungos agentes da blastomicose norte e sul-americana. Somos favoráveis a ésste ponto de vista, mas como bem demonstrou ALMEIDA (1946), não podemos aceitar a denominação genérica *Blastomyces*, pelas seguintes razões. O nome genérico *Blastomyces* foi proposto por CONSTANTIN e ROLAND para um fungo saprófita, que nada tem de semelhante com o causador da blastomicose norte-americana.

SACCARDO (1892) considera *Blastomyces* como nome ambiguo. Seria pois preferivel a denominação genérica *Gilchristia*, proposta por CIFERRI e REDAELLI, em 1934. *Gilchristia* é, contudo, posterior a *Paracoccidioides*, razão pela qual êste seria o unico gênero válido, com duas espécies: *Paracoccidioides dermatitidis e Paracoccidioides brasiliensis*. Não há duvida que existem aspectos muito semelhantes entre os dois fungos. Vejamos os caracteres diferenciais entre os mesmos.

1° — Aspecto microscópico — *Blastomyces dermatitidis* reproduz-se geralmente por brotamento unico. Esta forma de reprodução é também observada no *Paracoccidioides brasiliensis*, mas êste apresenta comunente a criptoesporulação, fato êste não evidenciado no primeiro cogumelo.

2° — Quanto às culturas, as do *Blastomyces dermatitidis* são mais luxuriantes, desenvolvendo-se com maior rapidez que as do *Paracoccidioides brasiliensis*. Ambas, conforme a composição do meio e a temperatura de cultivo, apresentam duas variantes principais: cotonosa e leveduriforme.

3° — As inoculações experimentais com o *Blastomyces dermatitidis* são facilmente realizadas, segundo CONANT e col. (1947) em cobaios e camundongos inoculados por via peritoneal. No caso do *Paracoccidioides brasiliensis*, somente a via testicular, em cobaios, é a preferivel, manifestando-se orquite especifica, sem tendência à generalização do processo.

4° — Quanto ao tipo de infecção que ambos os cogumelos determinam, há um ponto que deve merecer atenção: a grande frequência das lesões mucosas na blastomicose sul-americana, em contraste com o que se observa na sindrome de Gilchrist.

5°. — Finalmente, a questão da terapêutica.

No caso da blastomicose sul-americana, é incontestável o efeito terapéutico das sulfas, as quais até o presente momento continuam a não dar resultados práticos encorajadores nos doentes de blastomicose norte-americana.

As duas espécies — *brasiliensis* e *dermatitidis* poderiam ser colocadas em um mesmo gênero, mas não em *Blastomyces*, conforme procuramos demonstrar.

Resumo

No presente trabalho, o Autor defende a denominação genérica *Paracoccidioides*, assim como a espécie *brasiliensis*, de SPLENDORE, para designar o agente da blastomicose sul-americana. Refere que a espécie *brasiliensis* é perfeitamente válida e não fere nenhum dispositivo das regras internacionais

de nomenclatura botanica. Mostra que o binomio *Lutziomyces histosporocellularis* deve ser regeitado, por supérfluo e ilegítimo.

Summary

In this paper, the Author justifies the generic denomination *Paracoccidioides* and the SPLENDORE's species *brasiliensis* to designate the South-American blastomycosis agent. He states that the species *brasiliensis* is perfectly valid and does not infringe any item of the international rules of the botanic nomenclature. He shows that the binomial *Lutziomyces histosporocellularis* must be rejected as superfluous and illegitimate.

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Riferimento del genere *Paracoccidioides* agli Entomoftorali Imperfetti (Entomophthorales Coccidioidaceae)[1]

RAFFAELE CIFERRI

received Sept. 15th, 1950

Fu l'ALMEIDA che mise in luce, per la prima volta, la caratteristica morfologica fondamentale (non soltanto agli effetti della differenziazione del genere *Paracoccidioides* da *Coccidioides*, ma anche agli effetti della posizione sistematica del fungo) della genesi di ectosporule disposte a corona (cfr. ALMEIDA, 1930 *et seq.*). Non pare fu data importanza a questa insolita caratteristica morfologica, sinchè noi stessi (REDAELLI e CIFERRI, 1937) non la riconfermammo pienamente chiamando „criptosporulazione" il processo (e „criptosporule" le esospore) che ALMEIDA aveva denominato „gemmazioni multiple", mettendone in piena luce le sue peculiarietà.

Nel nostro studio del 1937 insistemmo particolarmente sull'importanza del fenomeno di questa criptosporulazione in rapporto alla posizione sistematica di *Paracoccidioides.* Però non sapemmo andare oltre la creazione di une famiglia delle Paracoccidioidacee in seno ai Chytridiale, con affinità presunte tra i Chitridiali meno evoluti.

Da allora è emersa una interessante analogia, che crediamo segnalata per la prima volta in una nota marginale di LANGERON (1945), tra *Paracoccidioides* e una Entomoftoracea pubblicata per la prima volta da COSTANTIN nel 1897: la *Boudierella coronata.* Poichè LANGERON si è limitato alla segnalazione dell'analogia tra *Paracoccidioides* e *Boudierella* (e sembra aver ignorato tutta la posteriore bibliografia su questo genere). abbiamo stimato opportuno approfondire le indagini in merito, dato che la *B. coronata* è una specie ormai ben nota, essendo stata ripetutamente isolata e studiata.

Il genere *Boudierella* fu creato appunto per la *B. coronata,* e, malgrado le ancora imperfette conoscenze, ascritto da CONSTANTIN agli Entomoftorali, per un fungo saprofita, ma con qualche sospetto circa una sua efficacia entonopatogena; sin d'allora CONSTANTIN ne notava le affinità con i generi *Basidiobolus* e *Conidiobolus,* mettendone in luce le differenze con *Boudierella* per la vita saprofitica e la formazione dei „conidi secondari" secondo una particolare modalità.

Poichè il nome generico di *Boudierella* era preoccupato e valido, SACCARDO e SYDOW (1889) lo mutarono in *Delacroixia.*

Pure in Francia GALLAUD (1905) reisolò la *Delacroixia coronata* (Cost.) Sacc. et Syd. e la studiò in maniera molto completa, dal punto di vista morfologico e morfogenetico, nonchè nel ciclo biologico, nei dati citologici e nelle caratteristische biologiche, riconfermando ed estendendo le osservazioni di COSTANTIN, e segnalandone le strette affinità con *Conidiobolus.* Neppure GALLAUD riuscì ad infettare gli insetti con questo fungo, che però sembrò avere una preferenza nel vivere su insetti morti.

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[1] Quest' argomento è stato oggetto di una nota presentata al III Congresso Internazionale di Microbiologia di Rio de Janeiro, Agosto 1950.

Un ulteriore reisolamento fu effettuato nel Nord America da GILBERT (1919), che considerò la *D. coronata* come un fungo entomoftoro, senza aggiungere nuovi dati importanti alla conoscenze della specie.

Pure nel Nord America la specie venne nuovamente isolata da MARTIN (1925) che la considerò come una nuova specie del genere *Conidiobolus*, denominandola *C. villosus* e confermando sostanzialmente i reperti precedenti.

Infine la stessa specie è stata reisolata tre volte ancora: da WHITE (1937) nel Nord America (sub *C. villosus* Martin); da KEVORKIAN (1937) in Puerto Rico (sub *Entomophthora coronata* (Cost.) Kevork.), ed ancora nel Nord America da COUCH (1939) (sub *D. coronata* (Cost.) Sacc. et Syd.). L'opinione di KEVORKIAN di fondere il genere *Delacroixia* con *Entomophthora* viene, a ragione, rifiutata da COUCH, per il fatto che in *D. coronata* la zigospore non sono conosciute. Il secondo-e più recente-reisolamento in Europa (Italia) si deve a CICCARONE (1950).

In ogni caso, *D. coronata* sembra essere un saprofita, specialmente associato a Basidiomiceti superiori, od al più un emiparassita di insetti, ancorchè KEVORKIAN lo consideri come un parassita delle Termiti.

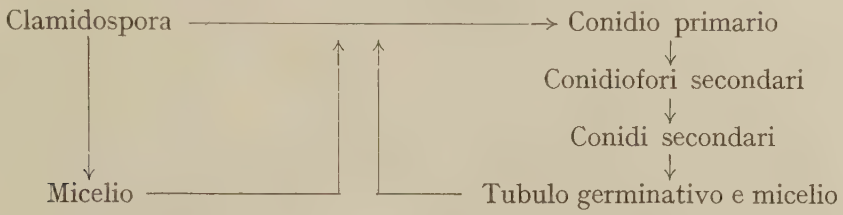
Delacroixia è un *Conidiobolus* di cui è ignota la forma zigosporica, che, come in quest'ultimo genere, sotto certe condizioni produce delle spore quiescenti (clamidospore, conidi primari, criptosporangi, ecc.) villose, per formazione di conidiofori secondari (sterigmi, spicule, basidi, ecc.), che possono generare dei conidi secondari i quali, germinando, riproducono il micelio. In sezione ottica, i conidi primari appaiono circondati dai conidi secondari come una corona o un ,,motore stellato d'areoplano'' molto caratteristicamente. Questa modalità conidica di moltiplicazione non sembra essere frequente nelle specie bene studiate di *Conidiobolus* (a partire de *C. Brefeldianus*), mentre — sotto certe condizioni — lo sarebbe in *D. coronata*, quasi a compenso della mancata produzione di zigospore.

In comune, dunque, tra *Delacroixia* e *Paracoccidioides* si ha l'assenza della forma perfetta di moltiplicazione (e dunque dello zigoto), e la modalità di riproduzione per conidi secondari (esosporule od ectosporule) generati e circondanti i conidi primari, carattere che ricollega entrambi i generi a *Conidiobolus*. Le differenze tra quei due generi sono d'ordine biologico, con le conseguenze connesse con la diversa modalità di vita delle *D. coronata* rispetto a *P. brasiliensis*. Mentre la prima specie è saprofita od al più debolmente parassita, con una certa tendenza all'entomofilia, la seconda è una specie parassita e patogena per l'uomo, con scarso adattamento alla vita saprofitaria in cultura. In conseguenza di ciò la morfologia della *D. coronata* in cultura è pienamente sviluppata, mentre quella di *P. brasiliensis* è regressa di fronte alla pur non ricca morfologia del fungo in attività patogena nei tessuti umani e degli animali recettivi inoculati. Si noti che anche in *D. coronata* i conidi primari formano i conidiofori e i conidi secondari solo allorquando quelli sono posti in condizioni di vita difficili, quale un mezzo secco e senza substrato nutritivo. In tal modo la formazione di conidi secondari in *D. coronata* apparisce come uno ,,stato di sofferenza'' del fungo, esattamente come in *P. brasiliensis* si formano i conidi secondari nei tessuti dei viventi recettivi, allorchè il fungo è in conflitto con i poteri difensivi dell'organismo, mentre in cultura normale formerà solo micelio e clamidospore non ulteriormente evolventesi. Infatti, i conidi secondari si osservano in tessuti umani parassitati dal *P. brasiliensis*, in casi spontanei di granuloma paracoccidioide; sono meno evidenti nei tessuti di animali relativamente recettivi in inoculazioni sperimentali. Per osservare l'esosporulazione ,,in vitro'', occorre porre il fungo in microaerobiosi o semi-anaerobiosi, come fecero, per la prima volta, REDAELLI e CIFERRI (1937), e quindi — con tecniche diverse, ma raggiungenti gli stessi scopi — Dowding (1948); Pereira—Filho (1948); Mackinnon e collab. (1949).

Apparentemente, solo quest'Autore prende in considerazione il significato della sporulazione che egli definisce „a ruota di timone'' (Mackinnon, 1950), in comparazione ad una forma analoga di moltiplicazione osservata nelle clamidospore di *Mucor*, che in mezzi liquidi produce una forma-lievito di riproduzione Comunque, il Mackinnon rifiuta l'idea di una ammissione di *Paracoccidioides* tra gli Entomoftorali, ma non ha indagato a fondo le analogie con questo gruppo, limitandosi ai pochi cenni di Langeron intorno a *Delacroixia*.

Possiamo dunque confrontare i cicli biologici dei due generi in questo modo:

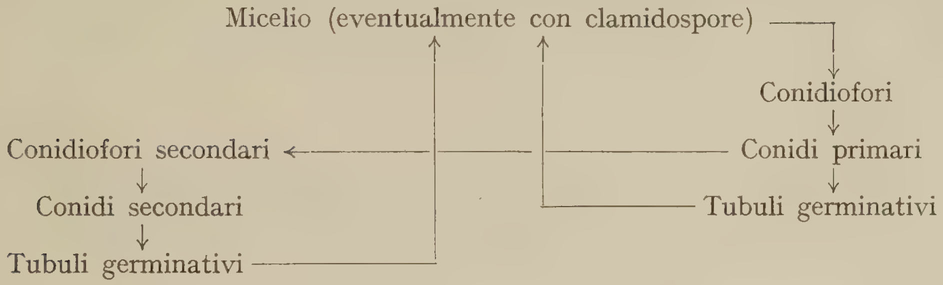
Ciclo vitale di *Paracoccidioides brasiliensis*


Clamidospora
Conidio primario
Conidiofori secondari
Conidi secondari
Micelio
Tubulo germinativo e micelio

(Ciclo della vita saprofitaria; in cultura normale)

(Ciclo della vita parassitaria; nei tessuti umani e sotto condizioni di sofferenza).

Ciclo vitale di *Delacroixia coronata*


Micelio (eventualmente con clamidospore)
Conidiofori
Conidiofori secondari
Conidi primari
Conidi secondari
Tubuli germinativi
Tubuli germinativi

(Ciclo della vita saprofitaria)

(sotto condizioni di sofferenza) (sotto condizioni normali)

In conseguenza di ciò, la famiglia **Paracoccidioidaceae** Cif. et Red. (Bull. Ist. Sier. Milan., **15**, 98.1936; Mem. R. Acc. d'Italia, Cl. Sci. Fis., Matem. e Natur., **8**, 595.1937) viene a situarsi tra i Chytridiales Imperfecti, assieme al gen. *Delacroixia*.

Le affinità fondamentali con gli altri generi degli Entomoftorali e le più salienti caratteristiche differenziali possono così riassumersi:

ENTOMOPHTHORALES

I) Riproduzione sessuale (zigospore) nota. Fam. **Entomophthoraceae**
  1) Modo di vita saprofitario.
    a) Micelio molto sviluppato. Genn. *Basidiobolus* Eidam; (incl. *Ichtyophones*); *Conidiobolus* Brefeld

2) Modo di vita parassitario.	
a) Parassita di Crittogame. Micelio poco sviluppato.	Genn. *Completoria* Lohde; *Ancylistes* Pfitzer
b) Parassita d'Insetti.	
α) Micelio molto sviluppato	Genn. *Entomophthora* Fres.; *Empusa* Cohn
β) Micelio poco sviluppato	*Massospora* Peck
II) Riproduzione sessuale ignota.	Fam. **Paracoccidioidaceae**
1) Modo di vita saprofitario o sub-parassitario in Insetti. Micelio molto sviluppato.	Genn. *Delacroixia* Sacc. et Syd.
2) Modo di vita parassitario. Micelio poco sviluppato. Parassita dell'uomo.	Genn. *Paracoccidioides* Almeida

Circa i rapporti intercedenti tra *Delacroixia* e *Paracoccidiodes*, si potrebbe ammettere che entrambi rappresentino due ,,phyla'' paralleli, specializzati per le modalità di vita, derivati da *Conidiobolus*: il gen. *Conidiobolus* degli Entomotorali perfetti e a vita saprofitaria, avrebbe dato origine al gen. *Delacroixia* degli Entomoftorali imperfetti, pure a vita saprofitaria, ma con qualche accenno di adattamento al parassitismo, e al gen. *Paracoccidioides*, egualmente degli Entomoftorali imperfetti, ma a vita parassitaria quasi obbligata.

Appare evidente che la linea evolutiva, sia delle Entomophthoraceae che delle Paracoccidioidaceae, è quella del passaggio dalla vita saprofitica a quella parassitica, e — in questa ultima — dalle forme provviste di micelio a quelle senza o con poco micelio. Anche nei riguardi della biologia del parassitismo, sono evidenti delle linee di specializzazione rispetto agli ospiti (Crittogame, Insetti ed uomo).

Summary

By comparison between *Paracoccidioides* and *Delacroixia*, the former genus is adscribed to the Family Paracoccidioidaceae (Imperfect Entomophthorales).

The relationship between the genera of Entomophthorales is discussed, together with the evolutive trend of the group.

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Una nuova specie di Cochliobolus

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(con tav. III)

(1.2.1950)

Nel corso di uno studio sulle malattie fogliari di alcune Graminacee dell' Africa orientale la nostra attenzione fu attratta da un caratteristico seccume che osservammo, tanto su esemplari di *Sporobolus affinis* A. Rich., raccolti presso Asmara (Eritrea) (leg. I. Baldrati, 12 Agosto 1914), quanto su esemplari di *Sporobolus Ruspolianus* Chiov. provenienti dalla Somalia britannica (5 Ottobre 1937), entrambi conservati nell'Erbario coloniale della Università di Firenze.

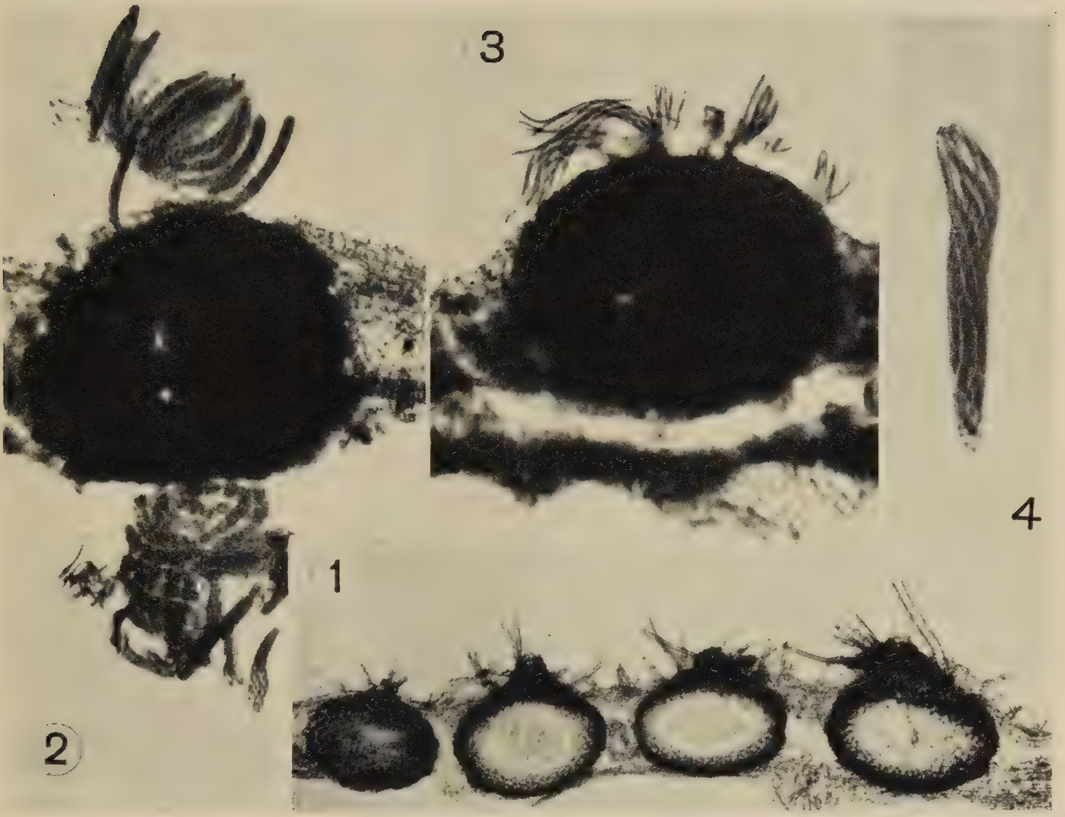
Tale seccume, particolarmente diffuso nelle foglie basali, ma non infrequente anche in quelle più alte, aveva generalmente inizio nella parte distale e spesso si estendeva gradualmente su tutta la lamina che, di conseguenza, assumeva una colorazione giallastra più o meno estesa, sfumante gradualmente nel normale colore verde dei tessuti finitimi.

Con l'ausilio del binoculare in tali macchie di secco potevamo osservare la presenza di numerosi corpiccioli neri, di regola posti negli spazi internervali, dapprima puntiformi ed immersi nel mesofillo, successivamente erompenti per sollevamento e lacerazione dell'epidermide ed assumenti allora una forma tendenzialmente ellissoidale, con l'asse maggiore disposto nel senso delle nervature (vedi Tav. II. fig. 1).

Tali corpiccioli che, dall'esame di numerose sezioni trasversali e longitudinali risultavano essere periteci, (vedi Tav. I. fig. 1) erano provvisti di un corto collo ostiolare, sul quale, e nella zona ad esso circostante, potevano osservarsi alcune setole brune.

Essi avevano dimensioni variabili intorno a *μ* 180—300 ⩔ 160—270 (nel campione somalo: *μ* 190—270 ⩔ 160—260 ed in quello eritreo: *μ* 180—330 ⩔ 200—270); avevano parete bruna, pseudoparenchimatica, costituita da cellule rotondeggianti o irregolarmente poligonali, del diametro medio di circa *μ* 10, più scure negli strati periferici e più chiare, sino a quasi ialine, in quelli più interni. Il collo, di forma largamente conica, misurava *μ* 40—50 ⩔ 40 (all'apice *μ* 32—40) ed era munito di un ostiolo del diametro di circa *μ* 12—14. Le setole erano brune, rigide, settate, lunghe da *μ* 100 ad oltre *μ* 170, larghe alla base *μ* 6—8, assottigliantisi all'apice (*μ* 4 circa), ove assumevano una colorazione più chiara.

Nell'interno dei periteci erano contenuti numerosi aschi cilindrici o lungamente clavati, diritti o leggermente curvi, misuranti *μ* 90—140 ⩔ 12—14, sessili o brevemente pedicellati, arrontondati all'apice ed attenuati alla base, con parete esile ed ialina (vedi Tav. I. figg. 2 e 4). Ogni asco conteneva 8 spore ialine, filamentose, appuntite alle estremità, misuranti *μ* 98—140 ⩔ 3—6, 4—6—settate, 2—3 volte spiralate l'una su l'altra, presentanti spesso al centro un piccolo nodulo intensamente colorabile con bleu lattico. Una volta espulse dagli aschi esse si distendevano a poco a poco e l'addensamento nodoso sopra indicato risultava particolarmente evidente.



Tav. I. *Cochliobolus Sporoboli* n. sp. 1) Sezioni di periteci in una foglia di *Sporobolus affinis* (× 93); 2) peritecio leggermente schiacciato sotto il copri-oggetto per provocare la fuoriuscita degli aschi (× 150); 3) peritecio con ascospore espulse (× 190); 4) asco (× 440).

Schiacciando un peritecio in acqua si aveva una scarica improvvisa di ascospore che si spostavano nel liquido a una certa distanza perdendo l'andamento elicoidale e divenendo più o meno falciformi (vedi Tav. I, fig. 3).

Frammiste agli aschi si osservavano numerose parafisi ialine, sottili, filamentose, leggermente più lunghe degli aschi (μ 120—150), del diametro di μ 1,5, plurisettate, spesso leggermente ingrossate all'apice, ove presentavano talvolta accenni di ramificazioni.

Per i caratteri sopra esposti l'Ascomicete in parola poteva agevolmente essere riferito al genere *Cochliobolus*, istituito, com è noto, nel 1934 dal DRECHSLER per accogliere fungilli simili agli *Ophiobolus*, ma da questi distinguibili a causa delle spore elicoidali o spiralate [1]), genere che inoltre, come appare dai lavori di DRECHSLER (1925 e 1934), di NISIKADO (1928), di DASTUR (1942), si distingue da quest'ultimo per essere in connessioni ontogenetiche con fungilli del genere *Helminthosporium*, anzichè con rappresentanti dei generi *Phoma* e *Chalara*.

Un ulteriore esame del materiale a nostra disposizione ci consentiva di osservare nelle stesse macchie di secco sopra indicate la presenza di una leggera lanugine olivacea, particolamente diffusa nella pagina inferiore. Esaminando al microscopio sezioni di foglie condotte in corrispondenza di tali macchie e pellicole di celloidina, — che preventivamente avevamo stesa su di esse onde raccogliere col minimo di alterazione possibile l'eventuale micelio o conidi presenti — osservammo la presenza di un fungillo che, anche nel nostro caso, poteva essere riferito al genere *Helminthosporium* (vedi Tav. II, fig. 2).

I conidiofori, di color bruno olivaceo più scuri alla base e più chiari all' apice, emergevano, isolati, o più frequentemente in gruppi di 2 o 3, attraverso le aperture stomatiche, misuravano μ 100—120 ed oltre di lunghezza ed avevano un diametro di circa μ 7—8 alla base, leggermente restringentesi all'apice (μ 5—6), erano plurisettati (distanza tra i setti μ 10—30) ed avevano andamento tortuoso. I conidi, come potevamo dedurre dalle numerose cicatrici dei conidiofori, erano inseriti su questi in posizione acropleurogena, avevano forma ellissoidale o cilindrica, leggermente allargata nella parte centrale, ed estremità ottusamente arrotondate, erano diritti, lunghi μ 22—40 (moda μ 32, media μ 32 ± 1,019), larghi μ 10—14 (moda μ 12, media μ 11,6 ± 0,27), generalmente 2—3-settati (più frequentemente 3), non ristretti ai setti, a parete liscia, bruno-olivacea, spessa circa μ 2.

Una certa differenza, riferibile con ogni probabilità alle diverse condizioni ambientali sotto le quali i fungilli si erano sviluppati, si notava nella forma e nelle misure dei conidi riscontrati negli exsiccata di *Sporobolus Ruspolianus* raccolti in Somalia, rispetto a quelli di *Sp. affinis* eritrei, che, nel primo caso presentavano una minore differenza nella lunghezza dei loro assi, avevano estremità più acute e erano provvisti di una parete alquanto più spessa (Tav. II. figg. 2 e 4).

L'esame accurato del materiale di cui sopra ci permetteva di osservare sulle foglie alcuni conidi germinanti con produzione di un tubulo germinativo apicale e di uno che si originava da una piccola zona posta nell'immediata vicinanza della cicatrice di inserzione (Tav. II, fig. 3). Ci trovavamo quindi di fronte ad un tipico rappresentante del sottogenere *Eu-Helminthosporium* di

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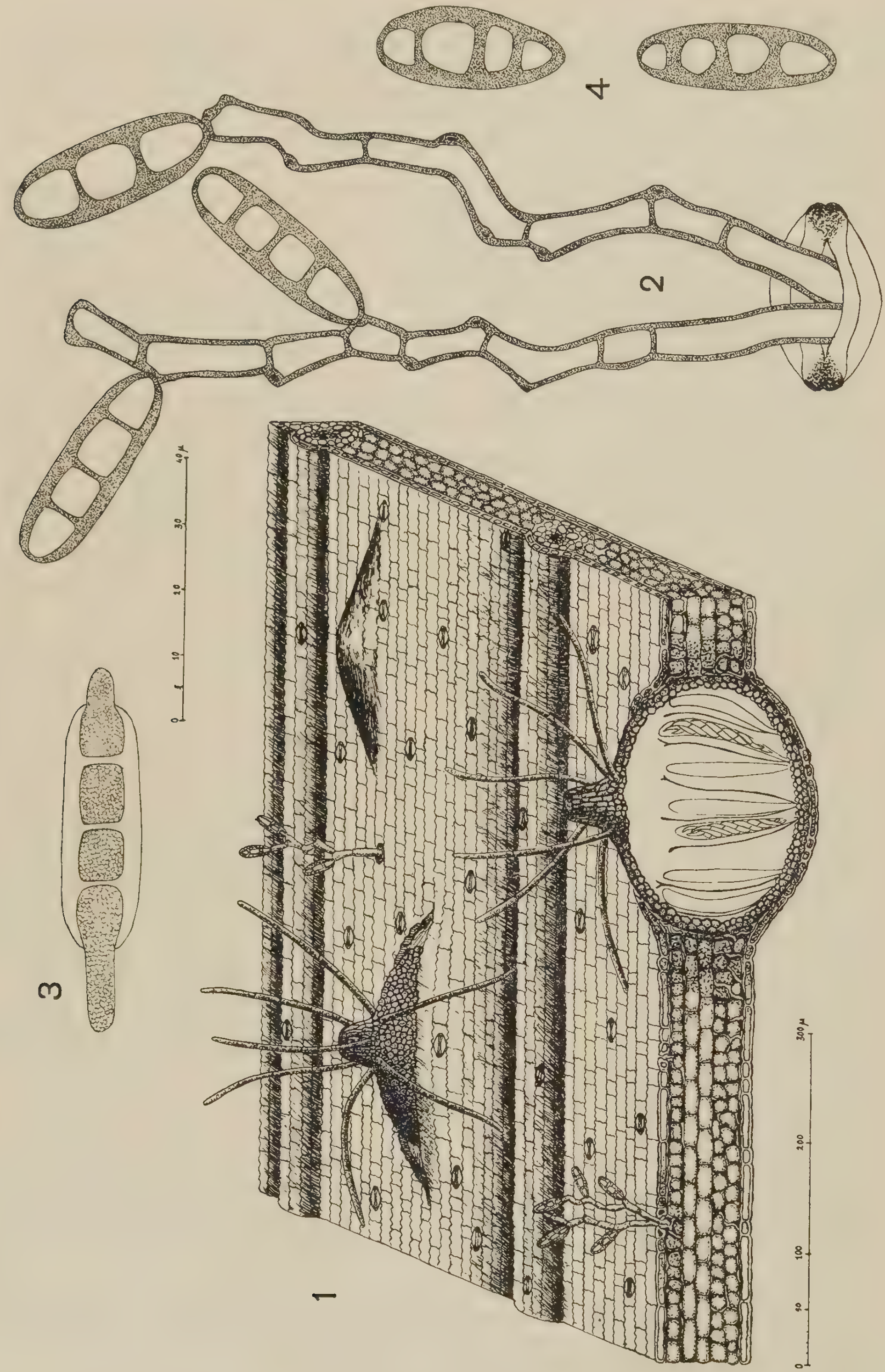
[1]) Reputiamo doveroso rilevare che, anteriormente all'A. americano, il NISIKADO (1928) aveva messo in rilievo le differenze morfologiche esistenti tra questi fungilli e i tipici *Ophiobolus* („*the morphological characters are not strictly in accordance with the diagnosis of the genus Ophiobolus*") al quale genere, in assenza di un altro più appropriato, aveva solo „*tentatively*" riferito le forme con ascospore elicoidali.


1
2
3
4
0 5 10 20 30 40 μ
0 50 100 200 300 μ

Tav. II. 1) Parte di foglia di *Sporobolus affinis* con periteci di *Cochliobolus Sporoboli* e con conidiofori e conidi della presunta sua forma metagenetica *Helminthosporium*; 2) e 3) Conidiofori e conidi di *Helminthosporium* da *Sp. affinis*; 4) conidi di *Helminthosporium* da *Sp. Ruspolianus*.

NISIKADO (1929) caratterizzato appunto, secondo l'A. giapponese, da conidi germinanti dalle cellule polari.

Sul genere *Sporobolus* non ci consta siano state segnalate specie di *Helminthosporium* diverse dal ben noto *H. Ravenelii* Curtis (in sinonimia di questo cadono infatti: *H. Hoffmanni* B. Mss. o *H. Hoffmanni* B. et C., *H. tonkinense* Karst. et Roum. e *H. crustaceum* P. Henn., cfr. DRECHSLER 1923) il quale, come si sa, in varie zone tropicali attacca con grande frequenza le infiorescenze di alcune specie di *Sporobolus* [1]) e, forse, quantunque DRECHSLER (loc. cit.) faccia in proposito le più ampie riserve, anche di altre Graminacee.

Di conseguenza abbiamo esaminato con particolare cura le infiorescenze del materiale a nostra disposizione, non solo delle specie sopra indicate, ma anche di diverse altre raccolte in Africa orientale, ma in nessun caso le abbiamo riscontrate attaccate da *Helminthosporium*.

Questo reperto, assieme alle misure dei conidi e conidiofori sopra riportate, pur non consentendoci un giudizio assolutamente sicuro, data la relativa scarsità del materiale a nostra disposizione, ci fa ritenere poco probabile il riferimento del fungillo da noi studiato a *Helminthosporium Ravenelli*, specie della quale già avevamo avuto occasione di osservare tipici esemplari provenienti dal Neotropico (cfr. CASTELLANI e CICCARONE 1939), le cui precedenti segnalazioni in Africa, in particolare su *Eragrostis*, come è stato altrove rilevato (CASTELLANI e CICCARONE loc. cit.), meritano ulteriore conferma.

L'avere riscontrato sulle stesse macchie fogliari i due fungilli sopradescritti lascia ritenere che essi siano fra di loro in rapporti ontogenetici. L'esistenza di questi è suffragata dal fatto che anche nel nostro caso i conidi della Demaziacea presentano la tipica germinazione bipolare [2]), costantemente osservata da diversi AA. (DRECHSLER, NISIKADO, DASTUR, loc. cit.) negli *Helminthosporium* dei quali sperimentalmente è stata dimostrata l'appartenenza al ciclo ontogenetico di specie di *Cochliobolus*.

Per quanto ci consta il genere *Cochliobolus* comprende attualmente le seguenti specie:

Cochliobolus heterostrophus (Drechsl.) Drechsl. in Phytopath., **24**, 953—983, 1934, (syn. *Ophiobolus heterostrophus* Drechsl. in J. agr. Res., **31**, 701—726, 1925), f. conidica: *Helminthosporium Maydis* Nisikado et Miyake, su *Zea Mays*;

C. Kusanoi (Nisik.) Drechsl. ex Dastur in Indian J. agr. Sci., **12**, 733, 1942, (syn. *O. Kusanoi* Nisik. in Jap. J. Bot., **4**, 108—109, 1928), f. conidica: *H. Kusanoi* Nisik., su *Eragrostis major*;

C. Miyabeanus (Ito et Kuribayashi) Drechsl. ex Dastur in Indian J. agr. Sci., **12**, 733, 1942, (syn. *O. Miyabeanus* Ito et Kurib. in Ann. phytopath. Soc. Jap., **2**, 1—8, 1927), f. conidica: *H. Oryzae* Breda de Hann, su *Oryza sativa*;

C. sativus (Ito et Kurib.) Drechsl. ex Dastur in Indian J. agr. Sci., **12**, 733, 1942, (syn. *O. sativus* Ito et Kurib. in Trans. Sapporo nat. Hist. Soc., **10**, 138—145, 1929), f. conidica: *H. sativum* Pammel, King et Bakke, su *Hordeum sativum* et *Triticum vulgare*;

C. Setariae (Ito et Kurib.) Drechsl. ex Dastur in Indian J. agr. Sci., **12**, 733, 1942, (syn. *O. Setariae* Ito et Kurib. in Proc. Imp. Acad. Tokyo, 6, 352—355, 1930), f. conidica: *H. Setariae* Saw., su *Setaria italica*, *S. glauca* e *S. viridis*;

C. stenospilus (Drechsl.) Matsumoto et Yamamoto in J. Pl. Prot., **23**, 9—14,

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[1]) In alcune regioni americane lo *Sporobolus indicus*, per la quasi costante presenza del parassita sulle sue infirorescenze, viene anche volgarmente indicato come „*smut grass*" o „*black seed grass*".

[2]) Questo tipo di germinazione manca, come è noto (cfr. NISIKADO 1929 e DRECHSLER 1934), nelle specie di *Helminthosporium* (sottogenere *Cylindro-Helminthosporium* di NISIKADO 1929) in rapporti ontogenetici con Ascomiceti feodictii del tipo *Pleospora* o *Pyrenophora*.

1936, f. conidica: *H. stenospilum* Drechsl. in Phytopath. **18**, 135—136, 1928, su *Saccharum officinarum;*
C. Tritici Dastur in Indian J. agr. Sci, **12**, 735—736, 1942, su *Triticum vulgare.*

Non quadrando la descrizione del nostro fungillo con nessuna delle diagnosi delle specie sopraricordate 1), anche a prescindere dal valore diagnostico che può attribuirsi alla diversa matrice, crediamo di poterlo considerare come una entità specifica a sè stante che indichiamo sotto il binomio: *Cochliobolus Sporoboli* e per la quale diamo la seguente frase diagnostica:

COCHLIOBOLUS SPOROBOLI nobis n. sp.

Peritheciis *sparsis, subglobosis vel ellipsoideis* (µ 180—300 ⩔ 160—270), *atris, coriaceo-membranaceis, ostiolo papillato* µ 12—14 *diam., hyphis sterilibus, brunneis, rigidis usque* µ 170 *et ultra longis,* µ 6—8 *crassis, praeditis;* ascis 8-*sporis, cylindraceis vel longe clavatis, sessilibus vel breve stipitatis,* µ 90—140 *longis,* µ 12—14 *crassis, hyalinis, tenuiter tunicatis;* ascosporis *filiformibus utrinque acutatis, media parte saepe noduloso incrassatis, hyalinis,* 4—6 *septatis, vehementer et regulariter spiraliter convolutis;* paraphysibus *numerosis, hyalinis, tenuibus pluriseptatis, ad apicem vix incrassatis.*

Forma conidica (Eu-Helminthosporium). Conidiophoris *solitariis vel* 2—3 *fasciculatim e stomatibus erumpentibus, tortuosis, simplicibus, olivaceo-brunneis, apicem versus saepe pallidioribus, ca.* µ 100—120 *longis,* µ 7—8 *crassis;* conidiis *acropleurogenis, elongato-ellipsoideis, utrinque attenuatis, rectis,* µ 22—40 (*moda* µ 32) *longis,* µ 12—14 (*moda* µ 12) *crassis,* 2—4, *saepius* 3-*septatis olivaceis, hyphis duabus polaribus germinantibus.*

Hab. in foliis *Sporoboli affinis* A. Rich., pr. Asmara (Erythrea, Afr. or.), leg. I. Baldrati, 12 ag. 1914, et *Sp. Ruspoliani* Chiov. (Graminaceae) Boundary, (Somalia britannica, Afr. or.) 5 ott. 1947.

Il nostro reperto arricchisce il numero delle specie di questo interessante genere, — alcuni rappresentanti del quale erano già stati segnalati in Africa sotto i generi *Ophiobolus* ed *Helminthosporium* (cfr. ad es. Hansford, 1943) — e ne estende notevolmente il limite boreale della area di distribuzione 2).

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1) Per comodità del lettore riportiamo nella seguente tabella le principali misure delle specie di *Cochliobolus* sinora conosciute in uno con quelle del ceppo de noi studialo, che indichiamo col binomio *C. Sporoboli*:

	Periteci	Becco ostiolare	Aschi	Ascospore	No. setti
C. heterostrophus . .	µ400 ⩔ 400–600	µ150 ⩔ 150	µ160–180 ⩔ 24–28	µ130–340 ⩔ 6–7	5–9
C. Kusanoi	µ300–350 ⩔ 300–350	—	µ130–170 ⩔ 14–18	µ140–170 ⩔ 5	6–8
C. Myabeanus . . .	µ370–760 ⩔ 370–780	µ95–200 ⩔ 55–110	µ142–235 ⩔ 21–36	µ235–468 ⩔ 6–9	6–16
C. sativus	µ530–770 ⩔ 340–470	µ90–150 ⩔ 80–110	µ110–220 ⩔ 32–45	µ160–360 ⩔ 6–9	6–13
C. Setariae.	µ240–500 ⩔ 220–315	µ60–125 ⩔ 50–110	µ130–150 ⩔ 22–32	µ200–315 ⩔ 6–7	5–9
C. stenospilus . . .	µ266–462 ⩔ 238–448	—	µ127–195 ⩔ 20–33	µ130–300 ⩔ 6–8	4–12
C. Tritici	µ220–597 ⩔ 172–406	µ53–236 ⩔ 56–124	µ80–228 ⩔ 13–34	µ125–301 ⩔ 4–8	4–12
C. Sporoboli. . . .	µ180–300 ⩔ 160–270	µ40–50 ⩔ 40	µ90–140 ⩔ 12–14	µ98–140 ⩔ 3–6	4–6

2) Da recenti studi di Baldacci (1948) *Helminthosporium Oryzae* Breda de Hann risulta assai frequente in Italia, ciò non di meno la sua forma ascofora *Cochliobolus Miyabeanus* (Ito et Kurib.) Drechsl. non sembra sia ancora mai stata segnalata nel nostro paese.

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(Dijon, 3 janv. 1874—Bourg-la-Reine, 27 juin 1950)

(avec tabl. IV)

Le 27 juin dernier, au numéro 15 de la rue Ravon, à Bourg-la-Reine, le Dr. Maurice Langeron mourait à l'âge de 77 ans. C'est dans cette maison qu'il habitait depuis près d'un demi-siècle que ses amis, ses fidèles, de nombreux visiteurs venus de tous les coins du monde renouaient avec lui les conversations du laboratoire de mycologie auquel il n'avait plus guère pu se rendre depuis qu'une fracture du fémur l'avait immobilisé de longs mois. Cependant, dans son cabinet de travail, parmi ses livres, ses fichiers, ses microscopes, ses souvenirs de voyage, il recevait chacun avec une patience infinie, écoutant d'une oreille attentive, prodiguant des conseils avec un tact parfait.

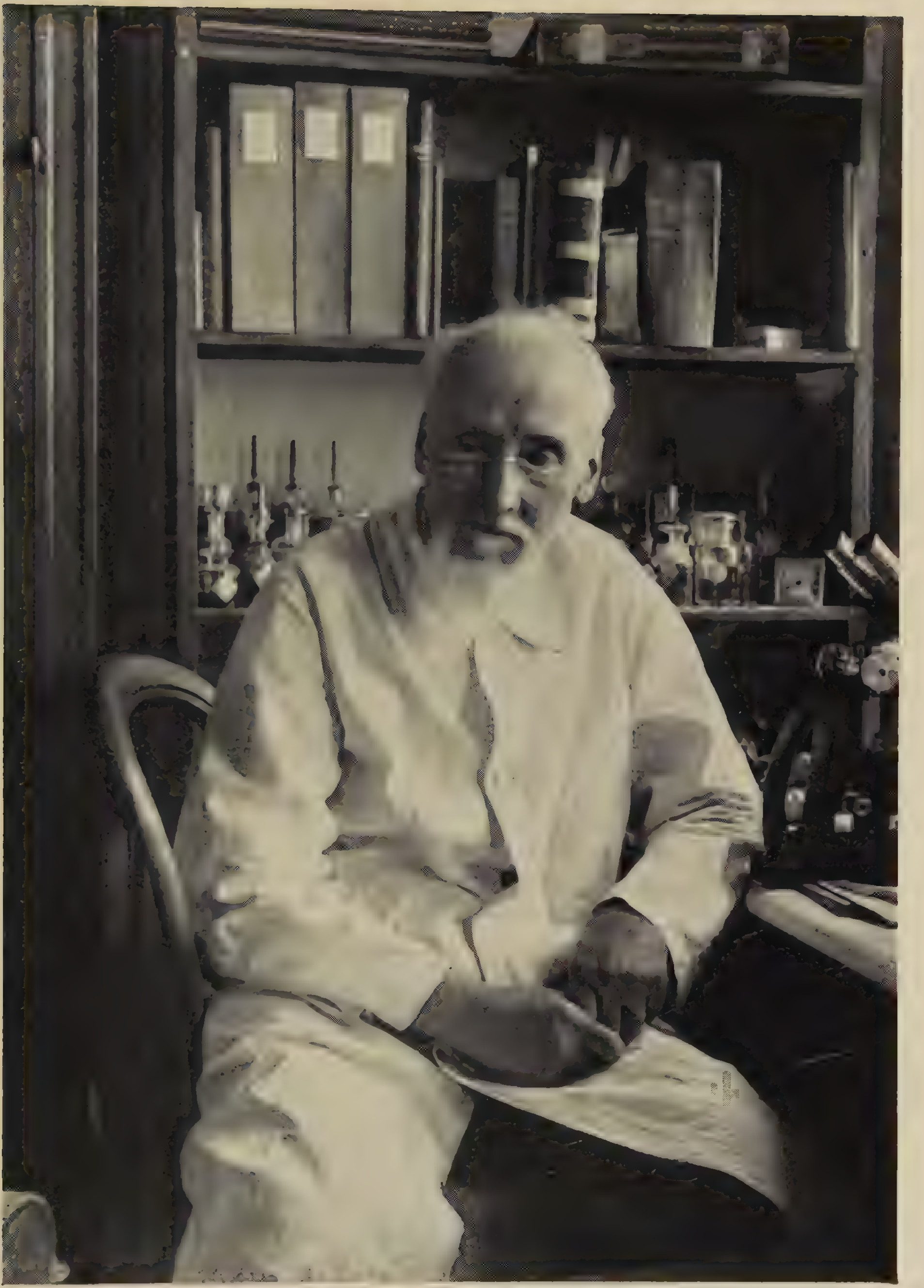
Ce ne fut qu'aux tout derniers mois qui précédèrent sa fin que l'activité du Dr. Langeron céda. En mars encore, nous passâmes avec lui une très longue après-midi: son esprit était intact, vif et agile, quoique la fatigue se marquât légèrement sur ses traits. Ainsi, jusqu'au bout, poursuivit-il sans faillir la carrière qu'il s'était choise tout jeune.

Né à Dijon en janvier 1874, Maurice Langeron suivit dans cette ville les cours du Collège St. François de Salle et en 1889—1890 passa avec succès ses deux baccalauréats. Il fréquente les cours de la Faculté des Sciences, passe son P.C.B. (alors P.C.N.) et en 1893 s'inscrit à Paris à l'Ecole de Médecine. En 1902, il est reçu Docteur en Médecine, sa thèse est consacrée au genre *Aleurites*. Ce n'était pas là cependant son premier travail. Une étude sur les mousses de la Côte d'Or, imprimée à Dijon lui avait valu, de l'Académie de cette ville, la médaille de vermeil en 1899. Une série de travaux de paléontologie végétale commencés en 1899 fut récompensée en 1912 par l'Académie des Sciences (Prix Sainton).

Cependant, l'année 1903 marqua le début de sa réelle orientation scientifique: la parasitologie médicale. Car c'est cette année-là qu'il devint l'assistant du Professeur Blanchard et plus tard celui du Professeur Emile Brumpt, tous deux parasitologues éminents. Il ne devait, jusqu'à sa mort, cesser de collaborer à l'activité de cet institut de parasitologie où il était entré 47 ans plus tôt. En 1913, il fut nommé chef de travaux de parasitologie à l'Institut de Médecine Tropicale et en 1929, il fut chargé de la direction du service de mycologie. Depuis lors, chaque année, il faisait une série de conférences consacrées à la mycologie.

C'est en 1913 que vit le jour la première édition du ,,Précis de Microscopie'' qui, plus qu'aucun autre de ses travaux contribua à répandre son nom dans le monde scientifique. Ouvrage modeste ses débuts, il devait en 1949, avec sa septième édition, prendre l'allure imposante d'un fort volume de 1430 pages. Ce ,,Langeron'' des laboratoires évoque la ténacité et la régularité de son auteur, qui ne laissait passer un jour sans accroître d'un rien la somme de ses connaissances. Nulla dies sine linea.

En 1926, avec Rondeau du Noyer, il publie un petit livre intitulé ,,Coprologie microscopique'' qui aura deux éditions. En 1945, son ,,Précis de Mycologie'' voit le jour. Ce dernier travail, résumé de toute sa vie, sera son testament scientifique. R. Y. Stanier, qui en fit la critique, écrivait: ,,In my opinion,



MAURICE LANGERON
1874—1950

the widespread use of this text in North American Universities, would make mycology a far more interesting and attractive subject than it commonly appears to-day".

En 1914, MAURICE LANGERON, engagé volontaire, fut affecté au Laboratoire de vaccination anti-typhoïdique du Val-de-Grâce et en 1917—1918 à une mission antipaludique.

Des missions nombreuses firent de cet homme de laboratoire un voyageur alerte: Tunisie (1910—1919—1920), Crète (1922), Amérique du Sud (1927), Espagne (1927), Yougoslavie (1931), Maroc (1936).

En 1937, MAURICE LANGERON devint Maître de recherechs de la C.N.R.S. Il appartenait à de nombreuses sociétés savantes et en 1936 fut vice-président de la Société de Pathologie Exotique.

Cet homme de sciences vivait une vie extrêmement retirée, consacrée entièrement à ses travaux. En dehors de la parasitologie en général et de la mycologie, il ne manifestait d'intérêt particulier pour aucune science, mais il en montrait pour toutes. Il ne restait pas étranger aux grands problèmes de l'évolution de l'Art et se reposait volontiers de ses études par la lecture des grands classiques français et étrangers. Montaigne et Erasme étaient ses dieux favoris. Très humble lui-même, il ne cessait de mettre en valeur dans sa conversation et dans sa correspondance ceux de ses élèves qu'il avait pu apprécier. Il ne pardonnait à personne la recherche des honneurs et son esprit caustique se laissait aller contre ceux qui font de la science un escalier conduisant à la notoriété. Ses travaux de mycologie en particulier sont imprégnés d'un souci considérable d'objectivité. Pour lui, non plus, il n'était pas nécessaire d'espérer pour entreprendre ni de réussir pour persévérer. Mais il persévéra et il réussit. Le travail était sa grande loi. Il avait coutume de dire: ,,Un homme intelligent qui travaille doit réussir". Mais réussir pour MAURICE LANGERON signifiait multiplier ses possibilités de travail.

D'une lettre que nous écrivit une de ses soeurs peu de jours après sa mort, nous reproduisons volontiers le passage suivant: ,,Si l'on recherche la part de ses ascendances dans ses qualités intellectuelles propres, je suis amenée à penser que son goût prononcé pour l'étude lui venait du côté paternel tandis que la direction de ce goût vers les sciences devait être hérité de la ligne francomtoise grand-maternelle où se rencontrèrent d'autres scientifiques. Du caractère comtois, il avait en effet la solidité, persévérance et une ténacité qui touchait parfois à l'entêtement. Du caractère bourguignon, il avait l'esprit vif et piquant, le trait parfois mordant, mais sans méchanceté".

Par la disparition de MAURICE LANGERON qui fut, pour nous, l'image de la ténacité et de la spiritualité françaises, le monde scientifique et la France elle-même ont beaucoup perdu. Nous exprimons à Madame LANGERON et à la Famille du Docteur MAURICE LANGERON notre vive sympathie.

R. VANBREUSEGHEM,
Institut de Médecine Tropicale Prince Léopold,
Anvers.

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N.B.: De 1918 à 1935, critiques des travaux de mycopathologie dans les Bulletins de l'Institut Pasteur.

The origin of some species of the genus Candida in non-pseudomycelium-producing anascosporogenous yeasts[1]

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(15.1.1951)

Variations in the morphology of yeasts have been noted for more than a quarter of a century, and the subject has been reviewed repeatedly (3, 4, 5, 18).

Some of the described variations were apparently responses to a change in environment, and when the cultures were returned to the original conditions, the original morphology again became manifest. In many cases, the changes described as permanent might have been due to the genetic makeup of the cells, since most ordinary yeast cultures consist of diploidal cells, which may be heterozygous for one or more characters. Consequently, meiosis and conjugation may well result in "variations" of some of the progeny from the parent diploidal culture. However, some yeasts are imperfect, lacking a sexual stage entirely, and are presumably haploid, although they may be sterile hybrids. There have been very few references in the literature to morphologic variations in imperfect yeasts. Obviously, irreversible variations in this group must be ascribed to mutations, and they cannot be regarded as due to the so-called "dual-phenomenon" or to nonsexual fusion of multinucleate cells, since these organisms are uninucleate, and hyphal fusions are not known to occur as they do in the Moniliaceae and the Dematiaceae. However, some of Roberts' (19) figures might be interpreted as evidence of nonsexual, rather than sexual, fusion in *Cryptococcus pulcherrimus*.

In this paper, only imperfect yeasts of the genus *Cryptococcus*[2] are being considered, and only one phase of variation is being studied, namely, elongation of cells to several times their diameter, so that when they hang together, as they usually do, they form a prominent pseudomycelium (and by the process of septation, often a true mycelium) from which blastospores sprout.

There have been scattered inferences in the literature to the effect that the above change does occur, but the authors have been unable to find any definite statement that unicellular imperfect yeasts may mutate to filamentous forms with blastospores. Henrici (4) noted this tendency in yeasts in general, and again posthumously (5), but he gives no data. His own work (15, 16,) in which variations in color were the principal ones studied, also showed more or less lasting morphologic variations, including production of a slight pseudomycelium in *Cryptococcus pulcherrimus*. Since publication of Punkari and Henrici's work, the organism which they studied has been claimed to be a perfect yeast (19, 25). However, it might be stated that one of the authors, after painstaking effort, was unable to find what he could regard as ascospores in any of several separate isolates, and LANGERON and WICKERHAM, in personal communications, also say that they are in no way convinced that *Cryptococcus pulcherrimus* produces ascospores, and interpret ROBERTS' findings differently than she did.

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[1] This investigation was supported in part by funds provided for biological and medical research by the State of Washington Initiative Measure No. 171.

[2] Whether *Cryptococcus* or *Torulopsis* (or both generic names) is valid is a question that is still undecided, and both names are in current use (20).

In this paper, "unicellular" isolates are considered those which fail to produce a definite pseudomycelium or a true mycelium in BENHAM (1) corn meal agar scratch plates, or which fail to produce a filamentous growth in beef peptone (pH 7.2) gelatin stab cultures. These are the two usual methods for distinguishing unicellular from mycelial yeasts, although except in North America, potato agar rather than corn meal agar is used. It is recognized that LANGERON and TALICE (6) were correct in regarding a pseudomycelial culture as unicellular, and the word "unicellular" is used here in the usual sense regarding yeasts, as meaning "lacking a pronounced pseudomycelium or a true mycelium". The edges of the "unicellular" colonies are smooth or slightly lobulated. Mycelial cultures are those which either produce the typical filamentous growth in gelatin stabs, called "fir-tree", or produce in corn meal agar, colonies which below or on the surface have very irregular edges with strands of mycelium proceeding from the streak, and penetrating into the substrate, and also have blastospores sprouting from the mycelium. We use the term "mycelium" here to include a prominent pseudomycelium.

Experimental results

Late in 1947, a large series of yeasts was isolated from various skin surfaces of about 200 persons at the University of Minnesota Hospitals, and the cultures were carefully purified by repeated replating on an acid medium. All isolates that produced a mycelium in corn meal agar scratch plates and beef peptone gelatin stabs were marked for further study, and the balance of the cultures were turned over to one of the writers, who retested all isolates by both methods mentioned. None of the tested cultures showed any mycelium, by either method. All pink nonfermenting yeasts (*Rhodotorula*) were discarded, as were all yeasts which formed ascospores on McKelvie's, Gorodkova's, or multivegetable agar (23).

Those cultures which survived the above selection processes were transferred to slants of modified SABOURAUD agar (5) and were maintained on that medium until 1949, when the cultures were again studied for spore formation and mycelium development. In addition to growing the cultures in beef peptone gelatin and in corn meal agar scratch plates, they were also grown under reduced oxygen tension in a thin layer of corn meal agar between a cover slip and a slide. To test for mycelial development, a series of 20 of the 131 cultures available was also subjected to rapid transfer in lithium chloride broth and corn meal agar pour plates, following the general procedure of FABIAN and MC CULLOUGH (3), for a period of seven months.

It was found that none of the 20 randomly chosen cultures subjected to the lithium chloride treatment ever did become mycelial, but that of the 131 original cultures in the group, a total of 17 had spontaneously dissociated to the rough form, as was evidenced by all three of the culturing conditions mentioned in the foregoing. Organisms that were originally nonfilamentous imperfect yeasts of the genus *Cryptococcus* had, apparently as a result of culture, dissociated so as to become morphologically identical with yeasts of the genus *Candida*. A comparison of the accompanying photomicrographs will indicate the extent of filamentation in several of the isolates. The rough characteristic, once attained, seems to be a constant one, and no reversions to the smooth form were observed.

TABLE I. *Extent of mycelial development in a collection of* 131 *yeasts, which lacked mycelium when they were isolated.*

Culture	Glucose Broth	"Fir-tree" growth in Gelatin stab	Filamentous growth in corn meal agar scratch plate	Filamentous growth in corn meal agar cover slip culture under reduced air tension
1	AG	++	++	++
2	AG	++	++	++
3	AG	++	++	++
4	AG	++	++	++
5	AG	++	++	++
6	AG	++	++	++
7	AG	++	++	++
8	AG	++	++	++
9	AG	++	++	++
10	AG	++	++	++
11	AG	++	++	++
12	AG	++	++	++
13	AG	++	++	++
14	AG	++	++	++
15	A	++	++	++
16	AG	++	++	++
17	AG	++	++	++
18	AG	—	—	+
19	A	+	—	—
20	A	+	—	—
21	A	—	+	—
22	AG	—	+	—
23	AG	—	+	—
24	AG	—	+	—
25	A	+	—	—
26	A	+	—	—
27	AG	+	—	—
28	AG	+	—	—
29	AG	+	—	+
30	AG	+	—	+
31	AG	+	—	+
32	AG	—	+	+
33	AG	—	+	+
34	A	+	—	+
35	A	+	—	+
36	A	+	—	+

#37—131 showed no evidence of pseudomycelium or mycelium on any of the media tested.
++ indicates pronounced and definite true mycelium or pseudomycelium.
(In gelatin stabs this was evident macroscopically.)
+ indicates slightly irregular edge of colony or very slight or doubtful mycelium. In gelatin stabs this was apparent only under the microscope (10 x).
A indicates acid production, G, gas production.

Discussion

The change of "unicellular" yeasts to filamentous forms by a spontaneous mutation in culture is not unique, and it is possible to show that this phenomenon is in the direction of changes that are usual in fungi and bacteria. With the latter, we usually term the variation "microbic dissociation", and call the change one from the "S" to the "R" form. HENRICI (5) posthumously published a chapter on variations in the lower fungi, and showed that among various fungi there was a general pattern of dissociation which may be outlined as follows:

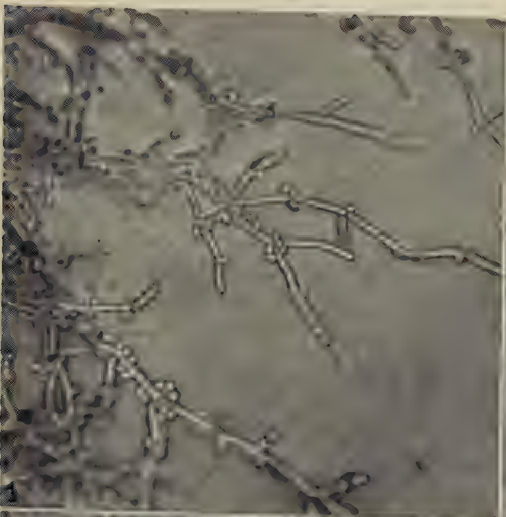
1. A. Unicellular yeast forms on all media → B. Unicellular yeast forms on surface, and mycelium with blastospores below the surface in certain media.
2. B. → C. Aerial or surface mycelium with blastospores or conidia.
3. C. → D. Sterile, wooley mycelium.
4. D. → E. Faviform growth, with low, flat, wrinkled, waxy mass of mycelium firmly adherent to agar, with no aerial mycelium or conidia.

Few if any fungi are known to dissociate the whole distance in the above scheme. Some cultures of *Blastomyces dermatiditis* go from A to D, and the so-called "black yeasts" regularly go very rapidly from A to C, finally appearing as molds of the nature of *Cladosporium* or *Torula* (sensu Saccardo). Some isolates of *Candida albicans* have been shown (8, 9, 10, 13, 14) to go from B to C, and the ringworm fungi, as is well known, often go from C to D, or C to E. Many molds go from C to D. It has been shown here that some asexual yeasts may go from A to B, or from A to C.

The pattern of this type of dissociation is nearly always in the direction of the formation of mycelial growth at the expense of reproductive cells, and hence the terminology of the German medical mycologists (degeneration) is particularly apt, for, except in the unnatural conditions of culture, the isolates as they "degenerate" become less and less reproductive, and the strain would therefore tend more and more to be crowded out in its struggle for existence in a natural habitat.

It will be noted that reverse variations have not been observed here, and it is tacitly assumed, but without much supporting evidence, that these changes do not occur. However, SUBRAMANIAM *et al.*, (22) have shown that changes in a reverse direction do sometimes occur in perfect yeasts, but it is evident that in *Candida* (8, 9, 10) they are very rare, as they are with most bacteria.

The close connection between *Cryptococcus* and *Candida* is evident, and some taxonomists might prefer to recognize only one inclusive genus, but this idea seems hardly practical, at least at present. When first isolated, the cultures are easily classified, and it is hardly the old laboratory strains of fungi that should be considered as setting the type any more than dried herbarium specimens of higher plants or fungi should set the standards for classification in their own groups. HENRICI (4) suggested that STELLING-DEKKER'S (21) lumping of mycelial and nonmycelial perfect yeasts in one genus, *Saccharomyces*, was more natural than LODDER'S (7) separation of the corresponding imperfect yeasts in two subfamilies. However, the classification of LODDER is useful, it is in current practice, and it was widely used even before she gave it its present standing — three compelling arguments in classifying Fungi Imperfecti, where a natural system of classification is rarely obtainable. The writers are inclined to prefer leaving LODDER'S system intact at present, to recognize



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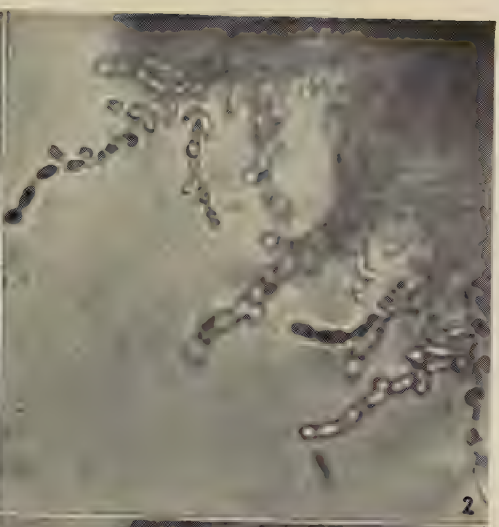

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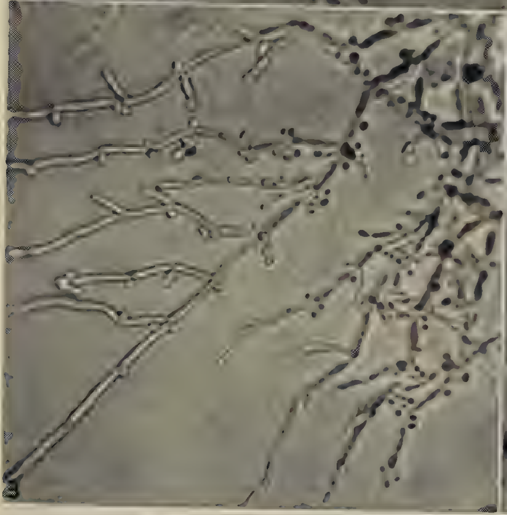

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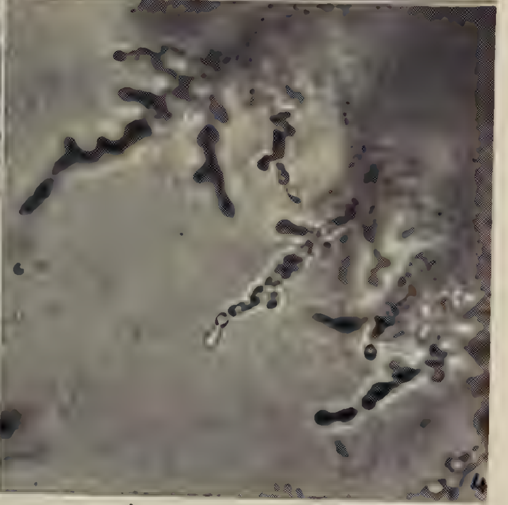

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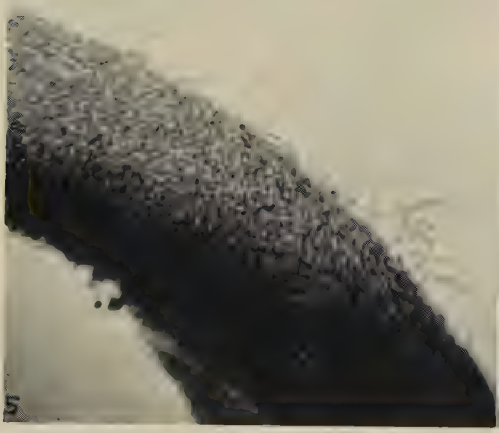

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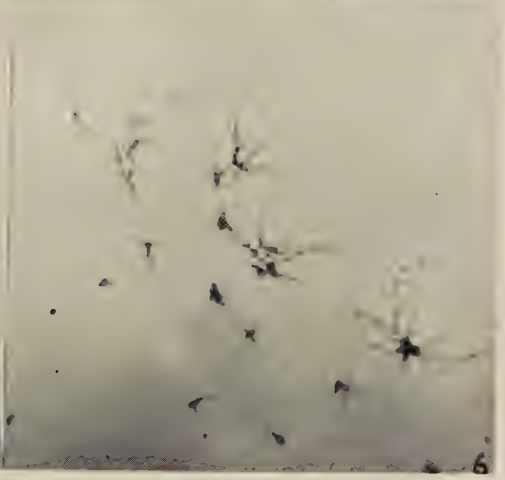

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Cryptococcus or *Candida* by the culture as it is, rather than by what it has been or will be, but to admit that *Cryptococcus* may frequently degenerate in culture into *Candida.* Further study with more data may justify a classification which will include, in one genus or in one species, cultures of anascosporogenous yeasts both with and without mycelium. This implies no opinion at present as to the mechanism of the change involved, and usage of the terms "degeneration," "dissociation", or "mutation" should be accepted in this light, since the phenomenon is merely being recorded, and not explained here.

Species of the genus *Candida* [1]) are obviously not all derived from *Cryptococcus.* Just as in many species, *Candida* is the rough form of *Cryptococcus* which is admitted to be derived from the perfect genus *Saccharomyces*, at least as far as the fermenting species are concerned (20), so is the pellicle producing *Candida Krusei* the somewhat more dissociated form of *Mycoderma cerevisiae*, which is likewise derived from the perfect genus *Pichia* or *Hansenula.* This very close connection between *Mycoderma* and *Candida Krusei* has been clearly pointed out (10, 11). It is also very probable from an observation of the same authors (12), that some non-fermenting species of *Candida* are colorless variants of *Rhodotorula.* In addition, the close morphological relationship between the perfect genus *Endomycopsis* and the imperfect genus *Candida* is generally recognized. Some species of *Candida* have not only a pseudomycelium, but also a true mycelium, including some species which grow entirely as unicellular yeasts on the surface of agar media. Such an organism is *Candida albicans.* This true mycelium, first carefully studied by WICKERHAM and RETTGER (24), is now generally admitted (2), and can be demonstrated by examination while growing in media such as corn meal agar designed to induce mycelial development. Thus the genus *Candida* in a natural system would probably cease to exist as a separate entity, and the species would be scattered among various other genera, one of them derived, not from the Ascomycetes, but from the Basidiomycetes. In an artificial system such as is generally used for imperfect fungi, *Candida* may well be retained, as stated above, at least for the present.

It is not only among the yeasts that a single pure culture belonging to a certain genus may produce a variant belonging to a genus in a different family. Molds belonging to the family Dematiaceae frequently produce saltants which if isolated from their natural habitat would have to be considered as belonging to the Moniliaceae. Although such a classification is far from a natural one, nevertheless the Saccardoan separation of the Moniliaceae from the Dematiaceae has been found useful and hence it has remained in spite of the fact that it is hardly a natural system. The knowledge that one genus may change in culture to another does not necessarily need to modify existing nomenclature at present, and HENRICI (4) may not have exaggerated when he declared that any yeast, if studied hard enough and long enough, will eventually be found to produce a true mycelium.

The writers are of the opinion that it will serve no useful purpose to transfer at the present time species of *Cryptococcus* (*Torulopsis*) to *Candida* unless the transfer is based on many freshly isolated as well as old laboratory cultures. We refer to such cases as DIDDENS and LODDER'S (2) transfer of *Torulopsis pulcherrima* to the genus *Candida.* It would not be surprising if it were found that some of the species of *Candida* recognized by DIDDENS and LODDER (2) were rough cultures of certain species of *Torulopsis* recognized by LODDER (7). Possibly both should have nomenclatorial recognition, just as we have a generic name for the perfect stage of certain fungi, but use another generic name for the imperfect stage. *Cladosporium, Fusarium,* and *Paecilomyces* are examples of common imperfect fungi whose perfect stages bear other names. It is becoming evident that even in the imperfect fungi, different growth phases bear different names, as *Cephalosporium* for the microconidial stage of *Fusarium.*

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[1]) The generic name *Candida* was antedated by *Syringospora* (17) which is clearly valid. However, a committee at the 1939 meeting of the International Microbiological Congress agreed to use *Candida* to replace the admittedly invalid name *Monilia*, which has long been used in another sense. Steps are currently being taken to have *Candida* validated by an International Botanical Congress as a *nomen conservandum.* Its use is currently all but universal.

Summary

One hundred thirty-one cultures of anascosporogenous yeasts isolated from the human body late in 1947 were all typical members of the genus *Cryptococcus* when examined in 1947 and early in 1948. No mycelium was produced in repeated tests in corn meal agar scratch plates or in beef peptone gelatin stabs. By 1950, 17 of the 131 cultures had definitely become morphologically identical with members of the genus *Candida,* showing typical pseudomycelial growth with blastospores sprouting from the mycelium on the above mentioned media. It is concluded that some species of *Candida* can be morphological dissociates of *Cryptococcus.* The status and the varied phylogeny of the genus *Candida* is discussed.

Sumario — El origen de unas especies del género *Candida* en levaduras imperfectas que no formaban seudomicelio.

Todos los 131 cultivos de una colección de levaduras imperfectas recientemente aisladas de la piel de seres humanos eran miembras típicas del género *Cryptococcus* en 1947. No producían ningún micelio, aunque lo buscabamos repeditas veces en gelosa de harina de maíz y en gelatina de carne y peptona. Ahora en 1950, 17 de los 131 cultivos se han convertido en formas identicas morfológicamente con miembras del genero *Candida.* En estas medias las 17 levaduras ya poseen un seudomicelio conspicuo de que muchas blastoesporas brotan. Se deduce que unas especies de *Candida* pueden ser formas de *Cryptococcus* producidas por disociación microbiana. Se trata del estado y de la filogenia multiple del género *Candida.*

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A leaf spot disease of *Panicum repens*, incited by a phycomycetous parasite

by

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(10.9.1950)

While collecting graminicolous smuts in the neighbourhood of Bangalore, South India, the occurrence of leaf spot disease on *Panicum repens*, L. growing along the fringe of a pond was noticed by the senior author. The *Panicum* plants were partially submerged during the rains, and later in the season became completely exposed to dry weather conditions. The leaf spots incited by the fungus were linear to lenticular, longer than broad, yellow in the initial stages and gradually turning yellowish-brown to chocolate-brown in colour. The spots were distinct and often coalesced with each other (Fig. 1). The largest spots measured 8 mm. long and 3 mm. broad. Even in later stages there was no tendency for the flaking away of the diseased portions, but the infected leaves showed tendencies to dry up much earlier than the healthy ones. Abundant infections were seen at one spot in 1946, and the whole area where the material was collected has since then been inundated. All attempts to recollect the fungus in the neighbourhood or any other place, have not been successful.

When the fungus was first collected, a casual microscopic study of the infected region showed the presence of numerous yellowish-brown, subspherical to deeply contorted chlamydospore-like spores (Fig. 2), which at first gave the impression that the fungus may be a smut species similar to *Entyloma* or *Melanotaenium*. But the spores were too irregular and lobed in shape, and further, were seen to be entirely intracellular unlike the smuts. In order to ascertain the identity of the fungus, the spores were scraped and placed on slides in moist chambers for germination. Instead of the development of a germ tube or a promycelium, the spores germinated by the formation of biciliate zoospores which escaped through a small neck-like pore, which gave the indication that the spore bodies were of the nature of sporangia and the fungus belonged to the group of Phycomycetes. A brief account of the studies carried out by the writers is presented here.

For cytological studies, the material was fixed in Allen's modification of Bouin's fluid, and microtome sections of 6 to 10 thickness were cut and stained with Heidenhain's iron-alum haematoxylin with orange G. as counterstain, or with Newton's iodine gentian violet. For germination studies, the spores were scraped from the host and mounted in droplets of water in hanging drop cultures and observations were made at frequent intervals.

The thallus is entirely intrammatrical (Fig. 2) and there is no mycelial stage of the fungus. Study of the early stages of development showed that the young thallus is a plasmodium with a multinucleate condition. As development proceeds, a thin membrane is formed, resulting in the formation of a young sporangium (Fig. 3). In some cells there were more than one thalli and hence, two or three sporangia were seen in later stages within a single host cell. There

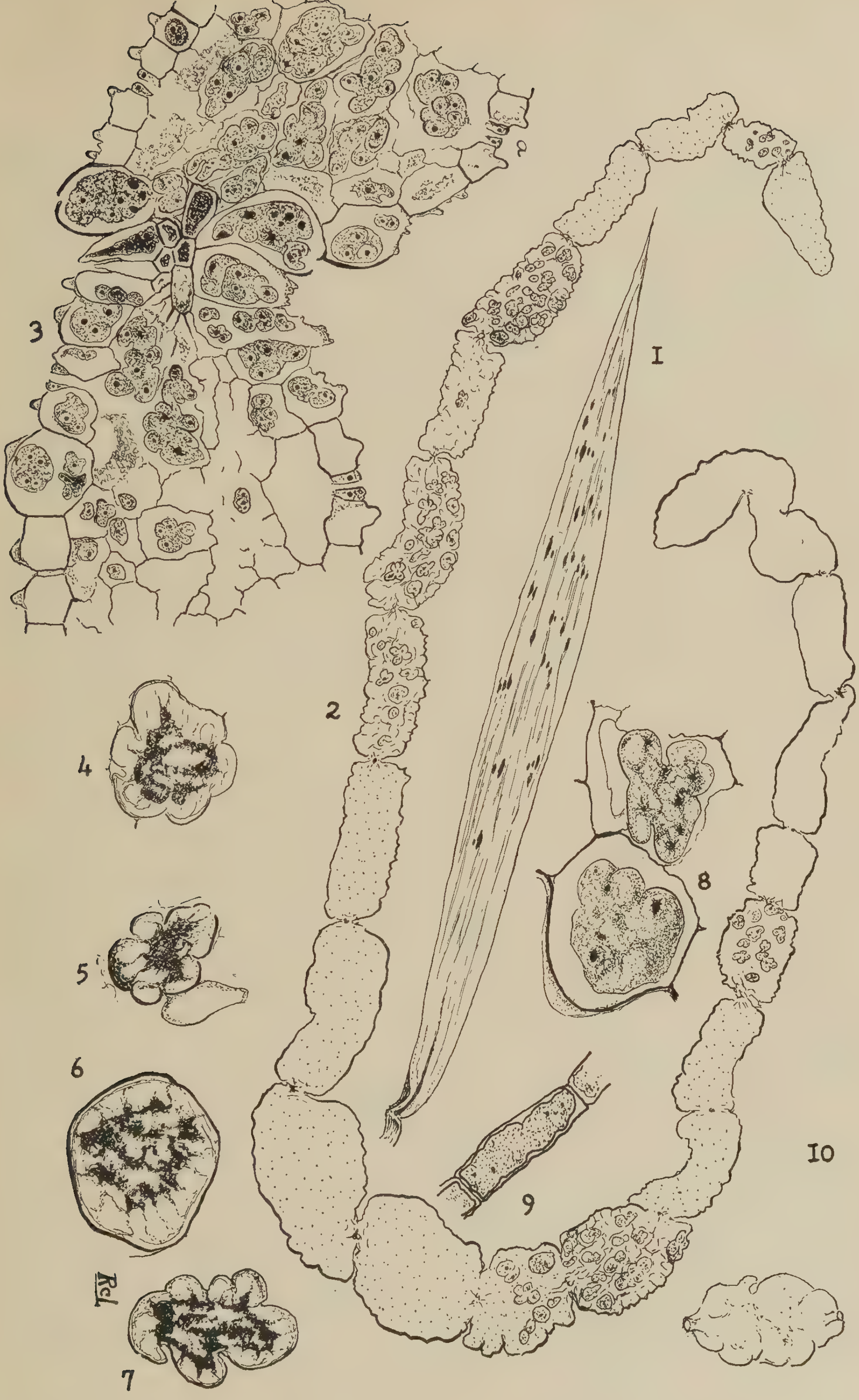

Fig. 1. Showing the infected leaf × 2.
Fig. 2. Section through the infected leaf showing the sori × 130.
Fig. 3. Enlarged view showing the stages of development of the sporangia × 620.
Figs. 4 to 8. Resting sporangia × 1320.
Fig. 9. Showing the sporangium within the mesophyll cell × 62o.
Fig. 10. Showing the exit tube formation × 1320.

was however no indication of the fragmentation of the thallus. The sporangia lie scattered either singly or in groups within the host cells of the infection patch. The thalli were small, deeply lobed and contorted in contour and were rarely spherical, (Figs. 6 to 8). Those within the epidermis were somewhat irregularly spherical with convolute foldings, while those within the spongy mesophyll, the cells of which are rectangular to cylindric with sinuous outline, the sporangia completely filled up the cells and possessed the same shape (Fig.9) Consequently, when such spongly cells are teased out, the sporangia of the same shape as the host cells escape out. In mature condition, the wall of the sporangia became thickened, assuming a pale cinnamonbrown to yellowish-brown colour and thus appearing similar to those of *Entyloma* species. Consequently the sporangia are not thin-walled and ephemeral, but are of the resting spore type.

As already stated, the sporangia have been germinated only at the time of collection of the material and all subsequent attempts to secure germinations using the old material have not been successful. The resting sporangia germinated by the formation of zoospores which escaped out by a short exit tube, (Fig. 10). The zoospores were somewhat reniform and laterally biciliate. Further role of the zoospores in reinfecting the host and other details are unknown.

As regards the identity of the fungus, the multinucleate plasmodial thallus developing into sporangia later on, and also lacking a mycelium, first indicated that it may be some olpidiaceous fungus among the chytrids. But the occurrence of a biciliate zoospore condition, soon dispelled this view, and further studies have pointed out that this fungus is best accomodated under *Petersenia* Sparrow, a member of the Lagenidiaceae.

The genus *Petersenia* was established by SPARROW (1934) to accomodate species of *Pleotrachelus* which were found to be biflagellate and not uniflagellate. All the species so far known are parasitic on marine algae (SPARROW 1943). The intramatrical thallus producing lobed or contorted thallus occupying one or more cells and developing zoospores which are biciliate and escape out through an exit tube or pore, are the characteristic features of the genus. The closely related *Sirolpidium* PETERSEN, differs from *Petersenia* in having a thallus which fragments at maturity to separate sporangia. In *Petersenia pollagaster* there is the formation of irregularly lobed resting spores of the type found in the *Panicum* fungus under study. KARLING (1942), however, doubts the assignment of the genus given by SPARROW for *Petersenia pollagaster*, and till the type of flagellation of the zoospores is confirmed, he prefers to recognise the previous identification of the fungus as *Pleotrachelus*. Therefore in his description of the genus *Petersenia* he states, "resting spores doubtful or unknown."

As regards the occurrence of the resting spores in the fungus under study, the following observations may be taken into account. While it is true that most of the species parasitising the aquatic plants can continue indefinitely by successive development of thin-walled sprangia and other spore structures, in a land plant exposed to adverse desiccating conditions, the formation of resting spores by the fungus parasitising the host should be expected. The presence of a primary plasmodial stage followed by the development of an irregular lobed and contorted thallus and resting spore, germinating by bicilizte zoospores discharged thorugh an exit tube are characters which indicate that the fungus on *Panicum repens* is a species of *Petersenia*. This, then, would be the first record of the genus *Petersenia* on a flowering plant and inciting leaf disease.

Petersenia Panicicola sp. nov.

Thallus intramatrical, unicellular, irregularly lobed and contorted, at first plasmodial, later forming resting sporangia, 17—27 × 10—15 μ. Resting sporangia pale cinnamon-yellow, medium thick-walled up to 1.7 μ. measuring 13.5—17 × 12—15 μ.; germinating by biciliate zoospores; exit tubes short and inconspicuous.

Hab. On the living leaves of *Panicum repens* L., Bannerghatta, Bangalore, India, leg. M. J. Thirumalachar, - 6 - 1946. Type deposited in the Herb. Crypt. Ind. Orient., New Delhi; Herb. I. M. I., Kew, England and in the Mycological division, U.S.D.A., Beltsville, Maryland ,U.S.A.

Thalli intramatrici, unicellulares, irregulariter lobati et contorti; primo plasmodii, demum sporangia quiescentia formantes. Sporangia quiescentia pallida, cinnamomeo-flava, magnitudinis 13.5—17 × 12—15 μ. semi-crassa, usque 1.7 μ.; germinantia per biciliatas zoosporas. Tubis exitus inconspicuis et brevis.

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Horse-hair fungus from Bengal

by

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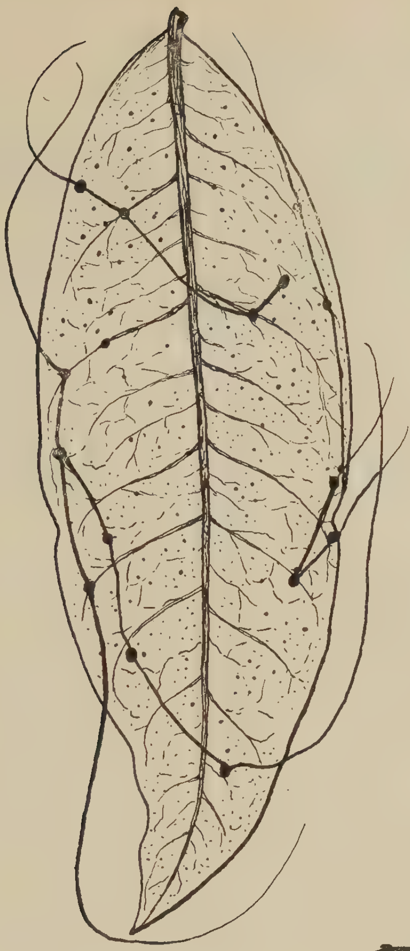
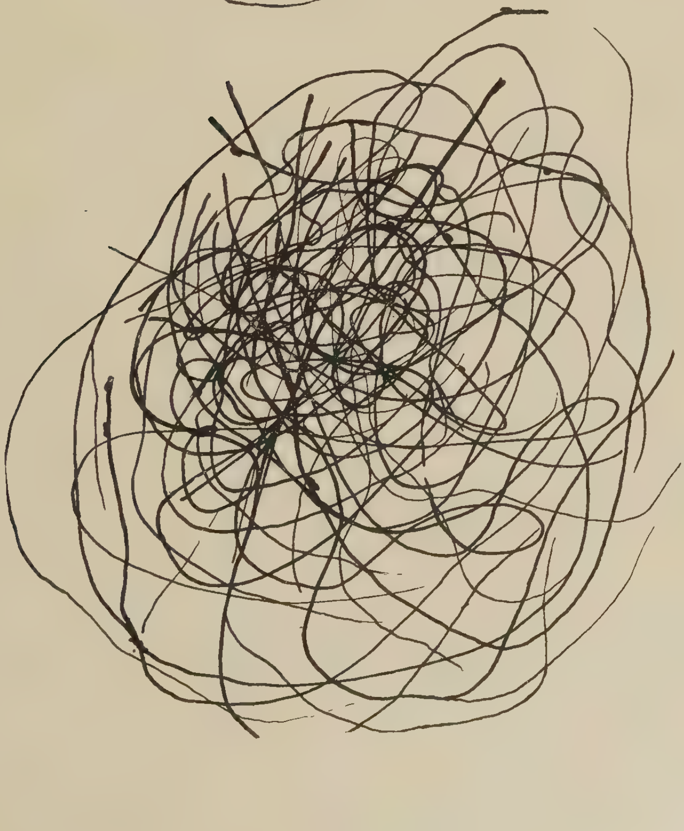
(16.9.1950)

The horse-hair fungus represents the mycelial stage of a *Marasmius* usually named as *Marasmius equicrinis* Mull. I have collections of such cords of mycelium from Sunderbans in 1921 and 1925 on leaves and branches of *Heritiera minor* ("Sundri" trees), from Chittagong (Chandraghona Kaptai Forest) in 1921 on bark of braches of *Dalbergia sisoo* ("Sisoo" trees), from Berkuda Island, Chilka Lake, Orissa, in 1922 on dead and dry leaves, and from a forest area close to Calcutta in 1937 on dead leaves and twigs. Dr. M. R. Henderson in 1927 collected black mycelial cords of *Marasmius* from dead branches and leaves in Singapore and kindly sent them to me, Dr. P. N. Mehra of East Punjab University, Amritsar, collected such black cords from *Qercus incana* in Mussorie hills (U. P. India) and Dr. R. N. Tandon has collected cords and small fructifications of *Marasmius* on bark of *Ficus religiosa*, recently growing within the compound of the Science College of Allahabad University.

The Bengal specimens are rhizomorphs of *Marasmius equicrinis* Mull. and *M. pangerangensis* P. Henn., both determined by Dr. G. Bresadola of Italy. The cords of mycelium are black and round; they are fairly strong and look like black horse-hair; externally they are covered by dead and brown peripheral hyphae and the internal hyphae are hyaline with distinct clamp-connections which are quite abundant. The cords run from tree to tree in bushes, hanging in festoons from the upper branches and remaining attached at intervals to leaves and stems by small brown discs (Text fig. 1). The whole course is aerial and a random one, being hardly connected with any mycelium on the ground. Many tiny fruit-bodies of *Marasmius* appear in the rainy season on the forest-bed when the leaves and twigs bearing the cords happen to fall to the ground. The fungus is not considered as a parasite but an epiphyte, causing no damage to the host trees; it merely grows on their surface and on dead leaves and twigs without deriving its nourishment from the living tissues. Sometimes, a tangled mass of dead leaves and twigs with the mycelial cords is found suspended in the forest. Petch (1923) has given a detailed description of the fungus *Marasmius equicrinis* Mull., and its fructification with appropriate figures in his book on 'the Diseases of the Tea bush' (pp 83—85). In Ceylon, late Mr. Petch recorded it on tea, *Hevea*, and nutmeg among cultivated plants while remarking that it is in the damp jungles where it grows over the various shrubs which constitute the undergrowth under the shade, and that this horse-hair Blight is known to occur in India, Ceylon, Java and Australia and probably is to be found throughout the eastern tropics.



SUNDRI LEAF
HORSE-HAIR BLIGHT



During the last Great War in December, 1943, this material was encountered by American soldiers in scrub oak forest during manoeuvres near S. W. Evans, Louisiana. The strands which extended from branch to branch often contacted the soldiers' faces as they plunged through the thickets and were thought by them to be spider web. The black cords were used by the soldiers for sewing buttons on coats and overcoats. Dr. Seaver was apparently a little surprised when it was sent to him at the New York Botanical Gardens for determination .It was a rare fungus for America; so far he could trace only one previous collection by Brother Arsene, an amateur collector, now deposited in the Farlow Herbarium at Harvard University. Dr. Seaver got the mycelial stage only (black cords) without any fructification. He has brought out a short description of 'the horse-hair fungus' with nice photographs of two bunches of black cords in Mycologia — vol. 36, 1944, pp. 340—342.

A taxonomic study of soil yeasts

by
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(Oct. 15th 1950)

Introduction

A variety of studies with respect to yeasts in the soil were made by Hansen (1881, 1882, 1890, 1895, 1896, 1902, 1903, 1905). He maintained that soil was the chief habitat of yeasts, though not the best, for only in soil could yeasts be found throughout the four seasons. Starting in the soil, yeasts are transported by wind, rain, animals, birds, or insects, to other breeding places, such as orchards, where they multiply extensively. Then in great numbers they return to the soil, where they have a rather modest existence until they are again transported to a sugary substrate.

Contemporaries of Hansen who made collaborating or correlating experiments with respect to the occurrence of yeasts in the soil are: Müller-Thurgau (1905); Wortmann (1897); Heinze (1905); Klöcker (1903, 1906, 1909); Ademetz (1886); De Kruyff (1908); and Boutroux (1881, 1883, 1884). Their observations verify those of Hansen. About twenty years later Starkey and Henrici (1927) isolated yeasts from soil, principally in Minnesota, to determine if these organisms live in this environment and if they were responsible for any major chemical transformation. They found that only 59 out of 89 soil samples contained yeasts and concluded that yeasts do not multiply in the soil. Starkey (1946) an unusual lipid synthesizing yeast from soil that was distinctly different from other yeasts studied for lipid synthesis.

Ciferri (1928) made a comparative study of the number and types of microorganisms occurring in soil in the Dominican Republic. He found a ratio of millions of bacteria; to thousands of molds; to a single isolate of yeast which was not identified. Lochhead and Farrel (1930), in Canada, isolated yeasts from apiary soils in order to determine if those present were able to infect honey. They found that only those soils in or near the apiaries contained the highly osmoduric types which might cause spoilage of honey. In a survey of yeasts occurring in soil in Germany, Nissen (1930) isolated 27 cultures. Over seventyfive per cent of his soil samples contained yeasts. Unfortunately his taxonomic treatment is difficult to interpret in line with the presently accepted procedures. A few of the isolates could multiply when seeded into suitable soils. He tested many yeasts to determine if they could use the common nutrients found in the soil. Starkey (1927).

All of these studies were made prior to the appearance of the newer taxonomic systems of Stelling Dekker (1931), Lodder (1934), and Diddens and Lodder (1942). The present study was made with the view of extending the information relating to the occurrence of yeasts in soils by the use of more recent techniques and procedures.

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[1] Present adress: Mac Donald Co., Saint Paul, Minnesota.

[2] I would like to express my appreciation to Dr. C. E. Skinner of Washington State College, for his help and guidance throughout this entire problem. I would also like to thank Dr. E. M. Mrak, of the University of California, Berkeley, for the privilege of working under his direction, and in his laboratory during the study.

Experimental Methods

The method of isolation for this survey included inoculation of large tubes of sterile enrichment liquor, then streak plating on consecutive days on acidic malt agar, kept under close observation to avoid mold overgrowth. When a colony of a rapidly spreading mold was noticed, its growth was inhibited by exposure of the moldy area of the covered petri dish to ultra-violet light for five minutes. The mold-free portion of the petri dish containing the yeast colonies was protected during the treatment by covering the top of the dish with a heavy sheet of paper.

The enrichment medium referred to above contained 1.5 % peptone, 3 % glucose, one half of an 18 gram commercial yeast cake and 10 ml. of commercial ,,V-8 Juice'' [1]) per liter. The pH of the medium was adjusted to 3.5 with HCl.

Soil samples were collected in the spring, before any fruit ripens and at a considerable distance from orchards or cultivated ground. The method of collection involved scraping the litter and leaves away with the top-soil to expose the soil and inch below the surface, and then removing soil with a sterile wooden spoon and filling into a sterile paper cup.

The procedures of identification used were similar to those outlined by STELLING DEKKER (1931), LODDER (1934) and DIDDENS and LODDER (1942) unless otherwise indicated.

Two additional sporulation media used were: vegetable agar of MRAK, PHAFF, and DOUGLAS (1942) and HENRICI's V-8 Juice sporulation medium as described by WICKERHAM, et al. (1946). All of the media containing wort (unfermented malt extract) were prepared in the manner described by STELLING DEKKER (1931), but for the fact that commercial dried malt ,,(Peerless'') was substituted for that prepared in the laboratory. Satisfactory comparative results are obtained with this medium. The fermentation media were prepared by dissolving 3 % of the desired sugar in 10 % yeast autolysate; the latter was prepared by autolyzing baker's yeast for 72 hours at 50° C. Durham tubes, rather than Einhorn tubes were used principally for convenience. For melibiose fermentation tests a prepared melibiose broth, as described by SKINNER and BOUTHILET (1947) was used.

Taxonomic Observations

One hundred and eighty cultures were isolated from eighty-four samples of soil. In most instances only the typical colonies from each plate or plates from a given soil sample were selected for study. When a single sample displayed two yeasts which were obviously different, both were isolated.

Twenty-five cultures were black-yeasts (probably *Pullularia*), the taxonomic position of which is at present in a state of confusion.

Twenty-five cultures appeared to be yeasts, but upon close examination were found unable to form blastospores and so were not included in the study.

Ten cultures died while in storage. These cultures turned brown on vegetable agar, and attained an appearance similar to certain species of *Pichia* on this medium.

The remaining one hundred and twenty cultures were studied intensively and diagnosed as to species and variety. Particular characteristics are indicated only in cases when cultures do not comply with the published description.

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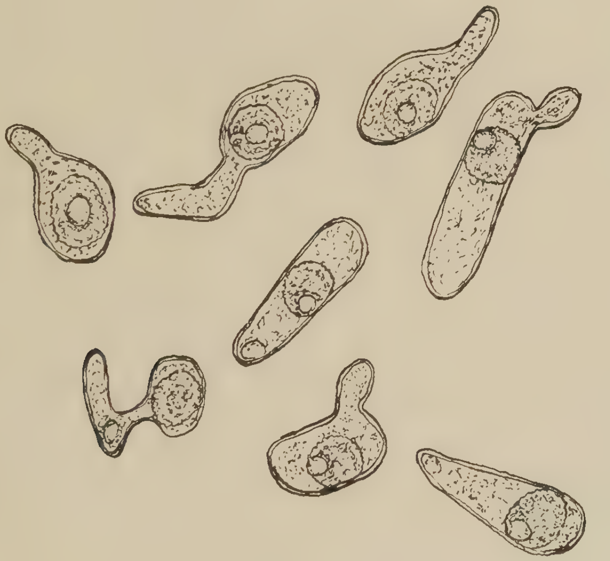
[1]) A commercial extract composed of tomato, celery, carrot, parsley, lettuce, beet, spinach, and watercress juices.

Ascospore forming yeasts: Of the cultures studies 48 formed ascospores whereas 94 were imperfects. The genera, species and number isolated are summarized in Table I. Thirty-six of the 48 perfect yeasts isolated are members of the genus *Hansenula* (including *Zygohansenula*). NISSEN (1930) and HENRICI (1927) also isolated a number of cultures of *Hansenula* from soil. Members of the genus *Hansenula* characteristically utilize nitrate whereas those of *Pichia*, *Debaryomyces* and *Torulospora* do not. This is of interest here since 75 % of the perfect yeasts isolated from soil during this study utilized nitrate.

All perfect yeasts except one conformed to the descriptions of BEDFORD (1942) for *Hansenula*, and STELLING DEKKER (1931) for the others. The isolates of *Zygohansenula californica* showed a definite tendency for the vegetative cycle to change from the haploid to the diploid condition. In other words, as the organism was held in culture, there was a gradual shift in the time of conjugation from just prior to spore formation, to just after spore formation.

The single culture of *Torulaspora rosei* sporulated slowly and then only on wort agar, and in giant colonies. The exception indicated above is considered a new species, and described below as *Debaryomyces mrakii* nov. sp. It was a difficult organism to place generically since the ascospores are very chracteristic of those of *Debaryomyces*, whereas the cells are not, as they vary in form from oval to sausage shape. Most species of *Debaryomyces*, on the other hand, have spherical or nearly spherical cells. In view of the spore and film characteristics and the failure to utilize nitrate, it was concluded that the organism is a new species and is included in the genus *Debaryomyces*. It is described below as *D. mrakii* nov. sp.



Debaryomyces mrakii nov. sp.: (Figure I). The cells in 24 hours in malt medium are long, sausage shaped, budding on ends + shoulders. The range of cell size is (2.5 × 7) — (7 × 20) u. A pellicle forms in 24 hours; it is yellow in color and heavily wrinkled.

The streak culture on malt agar is white to cream in color. The growth is heavy and is smooth with a semi-shiny surface. The asci are long and boat-shaped. Each ascus contains one spore which is spherical and warty, and contains a droplet of oil. Fermentation is evident in dextrose, galactose, sucrose, maltose and raffinose 1/3. Lactose is not fermented. No growth is found with nitrate as the sole source of nitrogen. Liquifaction of gelatine evident in 75 days. A pellicle forms in 4 days on ethyl alcohol medium.

Debaryomyces mrakii, species novum: In musto maltato, cellulae sunt magnae, plerumque cylindratae, interdum oblongae (2.5 — 7 × 7 — 20 u). Crassamentum et pellicula formantur. In asco saepe una ascospora invenitur. Ascosporae sunt plerumque rotundae, saepe cum tuberculis. Ascus est major et longior et forma similis botulo. Cultura in agaro maltato (post unum mensem, 17°) est flavida -alba, mollis, plerumque glabra. Nargo est glabra. Dextrosum, galactosum, maltosum, raffinosumque (⅓) fermentari possunt. Potest crescere in mediis in quibus aliquid ex eisdem saccharonis fons carbonis solus est. Crassamentum pelliculaque formantur in mediis in quibus spititus frumenti fons carbonis solus est. Sulfate ammoniae, asparagino, peptono, ureaque, uti potest. Gelatinum maltatum (post 2 menses 17°) liquefitur. Habitat in culturis ex solo in Minnesota, in America Septemtrionali.

Non-ascosporogenous yeasts: Of the 94 imperfect yeasts isolated and closely related organisms, 32 were members of the genus *Candida*, 25 *Pullularia*, 20 *Rhodotorula*, 16 *Torulopsis*, and one *Trichosporon*. The isolates of nonsporogenous yeasts conformed to the definitions of Diddens and Lodder (1942) for *Candida*, and Lodder (1934) for *Torulopsis* and *Rhodotorula*. The single isolate termed *Trichosporon cerebriformis* was similar to that described by Ota, Jap. Jour. Dermatol & Urol 28, (1928) (cited from Diddens and Lodder (1942)), and formed both blastospores and arthrospores, as is characteristic of the genus. Apparently the culture studied by Diddens and Lodder had lost its ability to form blastospores, since this change appears to occur in the genus *Thrichosporon*.

The so-called black yeasts are included in the genus *Pullularia* as defined by Berkhout (1923) for *Dematium*, and are probably members of the species *P. pullulans* as defined by Jorgensen, Hansen and Lund (1939). Since these organisms have not been studied as intensively taxonomically as most other yeasts, it is difficult to handle them systematically. In working with these organisms it was noted that the cultures were creamy to slightly pink, and typically yeast-like during the early stages of development. Later the cultures turned slightly greenish and finally black. The time required for this series of changes in colony color varied considerably with the medium. In fact those grown on media low in carbohydrate never turned black, whereas those grown in the presence of sugars darkened very rapidly. Cultures that remained creamy pink were classified as *Trichosporon pullulans*, although these same cultures also darkend when excess sugar was added.

During the taxonomic investigations on these black yeasts some observations on the character of the pigment were made. In the first place, the pigment never began to develop while the culture was actively growing. If a continuous source of nutrients were made available pigmentation was retarded. Semi-anaerobic conditions or a medium high in reducing substances such as ascorbic acid inhibited the pigment formation.

There is even some doubt in the mind of the author as to character of the pigment itself. When an attempt was made to isolate the black compound its complete resistance to solution made it possible to concentrate the pigment by repeated hydrolytic washes with NaOH and HCl, which removed most of the cell debris. However, micro-Kjehldahl analysis of the more purified fractions indicated that the nitrogen content was dropping too low to indicate a Melanin. It is the authors opinion that the pigment is a furfural black or some such compound derived from the breakdown of carbohydrate. These observations support the view of Mrak and Phaff (1948) that *Pullularia* should be included in the yeasts, rather than excluded because of the presence of a black pigment (DIDDENS and LODDER, 1942).

Although a large number of the ascospore forming yeasts isolated were able to utilize nitrate only five of the imperfects, (*Torulopsis albida* and 4 *R. glutinis*) possessed this ability. Imperfect cultures of *Hansenula* (nitrate users) were not apparent, as might be presumed.

Table I. Genera and Species of Yeasts Isolated from Soil

Genera and Species	Number of Cultures Isolated
Ascosporogenous Yeasts	
Hansenula saturnus Klocker.	23
,, *anomala* Sydow	4
Zygohansenula californica Lodder	9
Pichia membranaefaciens Hansen	4
,, *belgica* (Lindner) Dekker.	4
Debaryomyces membranaefaciens Naganishi . . .	2
,, *mrakii* Nov. Sp.	1
Torulaspora rosei Guilliermond	1
Non-ascosporogenous Yeasts	
Candida monosa (Kluyver) Diddens and Lodder	20
Candida tropicalis var. *lambica* Diddens and Lodder.	5
,, *robusta* Diddens and Lodder	3
,, *pseudotropicalis* Berkhout	2
,, *guilliermondii* Langeron and Guerra . .	1
,, *zeylanoides* Langeron and Guerra . . .	1
Rhodotorula mucilaginosa Harrison.	10
,, *minuta* Harrison	5
,, *glutinus* Harrison.	4
,, *pallida* Lodder	1
Torulopsis candida (Saito) Lodder	8
,, *flavescens* (Saito) Lodder	6
,, *uvae* (Pollacci and Nannizzi)	1
,, *albida* Saito	1
Trichosporon cerebriformis Ota.	1
Pullularia pullulans?	25
Total. . .	142

It is somewhat surprising that at least a few cultures of *Sporobolomyces* were not encountered. These organisms occur so commonly on decaying organic matter such as leaves. It is, however, possible that the cultures of *Sporobolomyces* had lost their ability fo form basidiospores, and were either classified in the genus *Rhodotorula* or in *Torulopsis* if they had also lost the ability to form pigment.

Summary

Eighty-four samples of Minnesota soils were collected in the spring of the year. All samples yielded yeasts, and a total of 180 cultures were isolated of which 117 were studied taxonomically. Twenty-five cultures were black yeasts. Approximately one-third of the isolates were spore forming yeasts and members of the oxidative and film forming genera *Hansenula, Pichia*, and *Debaryomyces*, of which a new species (*D. mrakii*) has been described. Not a single culture of *Saccharomyces* was isolated. Perhaps later in the season when fruit was abundant members of this genus would have been present.

The greatest number of imperfects belonged to the genus *Candida* (32 cultures), although *Pullularia* (25 cultures), *Rhodotorula* (20 cultures), and *Torulopsis* (16 cultures) were well represented.

The cultures of *Pullularia* darkened very slowly if at all, in the asbence of excess sugar. Under these circumstances they were very similar to *T. pullulans*.

A single culture of *Trichisporon cerebriformis* was isolated. The culture isolated formed both blastospores and arthrospores.

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A Clinical, Pathological, and Mycological Study of a Fatal Case of Histoplasmosis in an Infant

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The first successful cultivation of *Histoplasma capsulatum* Darling was reported by DeMonbreun (1934). The life cycle of this fungus was initially worked out by DeMonbreun (1934) and Conant (1941). Parsons and Zarafonetis (1945) reviewed all the known cases up to that time. Iams, Tenen, and Flanagen (1945) reviewed Histoplasmosis in children, reporting on 21 children under 13 years of age, 15 of whom were less than 16 months old.

The onset of the disease in children is usually insideous, with general symptoms of fever, pallor, diarrhea, failure to gain or actual loss of weight, difficulty on feeding, chronic cough, irritability, abdominal distension, cyanosis with feeding, hepatomegaly, splenomegaly, lymphadenopathy, purpura, anemia, and leukopenia.

Diagnosis is occasionally accomplished ante mortem. Of the 21 cases reviewed by Iams and his workers, diagnosis was established in one case by splenic puncture and in three others by biopsy of the bone marrow. Dodd and Tompkins (1934) also found the characteristic yeast cells in the mononuclear cells of a blood specimen taken during the life of a six-month-old infant.

The manner in which infection occurs is not understood. The possibility that dogs and other animals may be carriers of Histoplasmosis (DeMonbreun, 1939) cannot be disregarded. In a few instances the fungus has been recovered from family pets[2]) (Thuringer, 1944). Emmons, Bell, and Olson (1947) examined 1,620 animals in a rural county in Virginia where 4 human and 3 canine infections had occurred and isolated *H. capsulatum* from one mouse and 10 rats. This discovery suggested the possibility of rodents being a natural reservoir of the disease.

Since the average duration of the disease is about 3 months (Iams, Tenen, and Flanagen, 1945), intrauterine infection should be suspected in those cases in which symptoms develop before the patient is 3 months old. The culture used by Conant (1941) in working out its life cycle was isolated from the bloodstream of a 3-month-old infant 24 hours before death.

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[2]) McClellan (1949) has found Histoplasma in an 18-month-old female cocker spaniel. This dog was born in a kennel and purchased as a pup by a physician. She was raised under nearly ideal conditions and rarely had the opportunity to leave the premises of the physician for the rest of her life. The diagnosis of Histoplasmosis was made from a peripheral blood smear. Thereafter the animal was sacrificed and the organisms were found, both by culture and in histologic preparations, in all the organs examined, including the heart, kidneys, and gastro-intestinal tract.

The following case has been reported in order to record the clinical and pathological findings in a case of fatal Histoplasmosis in a 3-month-old infant, to point out the possibility of diagnosis of this disease during life by liver biopsy and smears of biopsy material, and to record a mycological study of *H. capsulatum.*

History and Hospital Reports

This case concerns a 3-month-old white, female infant from Jessamine County, Kentucky. She was two weeks premature at birth, weighing 4½ pounds. She was breast fed for a short period, then tried on various formulae of evaporated milk and cows milk, but she did poorly on all, reaching a maximum weight of only 6½ pounds.

The bowel movements were always copious, soft, and of a frequency of 4 to 8 times daily. When the child was approximately one month old her parents noticed thah the abdomen was increasing in size. On the day previous to admission the child exhibited symptoms of grunting respirations and fretful crying. At this time the mother noticed a discolored blue area over the right side of the abdomen.

The family history revealed that the mother, who had been married twice, had had by her first husband 14 children, all of whom had died before the age of 3 months. Three childern by her second husband are living and apparently well. The maternal grandmother had cancer and the mother has a goitre.

The infant was admitted to the Good Samaritan Hospital in Lexington. Kentucky, November 29, 1929.

Physical examination revealed an emaciated, dehydrated, white, female infant approximately 3 months of age and obviously in acute distress. She weighed 5 pounds and 13 ounces. The eyes were sunken; the pupils were regular and reacted to light and accommodation. There were no strabismus and no nystagmus. The eardrums were red, but not bulging. The mouth and throat showed no lesions.

The heart was regular, with a rate of 100 beats per minute, and there was no enlargement. The heart sound were of fair quality and intensity, with no murmurs. A few fine rales were heard over the base of both lungs posteriorly. The respirations were 20 per minute; temperature was 101.6 F.

The abdomen was greatly distended, and there was a peculiar blue coloration in the right upper quadrant extending down to the umbilicus. This area, approximately 3 × 4 inches, was not sharply defined. The liver and spleen were markedly enlarged. No other definite masses were palpable in the abdomen.

The skin was dry and there was little, if any, subcutaneous fat present. On the legs were two healed lesions resembling impetigo. The genitalia, reflexes, bones and joints showed no abnormalities.

Laboratory examinations revealed the urine to be yellow, acid in reaction and negative for sugar, albumin, and actone. Mircroscopic examination revealed occasional red blood cells, and 6—8 white blood cells per high power field. The blood showed 3,150,000 red blood cells per cubic mm, with a hemoglobin of 8.7 gm per 100 cc, or 56.5 %. The white blood count was 6,000 per cubic mm, with polymorphonuclear leukocytes 26 % and lymphocytes 74 %. No marked variation was noted in the red blood cells.

In the hospital the child was given fluids and Metranigen by mouth; these were taken eagerly and retained. The stools continued frequent, loose and greenish, and were not noticeably foul. The child was restless and cried out with pain. She was given phenobarbital as a sedative.

A tuberculin patch test and a 1—1000 dilution of histoplasmin injected subcutaneously produced negative reactions. An *x*-ray of the chest revealed diffuse mottling and heavy infiltration throughout both lungs. A liver biopsy was obtained by the needle-punch method.

On the second day of hospitalization the child's condition became much worse and she was placed in an oxygen tent and given stimulating drugs. The legs became cyanotic and edematous. On the morning of the third day in the hospital she became greatly cyanotic, with rapid grunting respirations, and expired after being in the hospital a total time of 52 hours.

Autopsy Report

An autopsy was performed on the unembalmed body before the results of the liver biopsy were available. The abdomen was quite distended, and over the right hypochondrium there was a hemorrhagic area, 5 cm in diameter. Near the center of this discoloration there was evidence of needle puncture. The right arm was edematous and slightly discolored, and there was slight edema in each lower leg.

Internally, the pleural cavities were smooth. The lungs were firm and mottled, showing many grayish-yellow flecks and some larger confluent lesions scattered over the surfaces. On section, the parenchyma of the lungs appeared greatly consolidated. The hilar lymph nodes were enlarged and gray. Some of the mesenteric lymph nodes appeared caseous. The heart and the large blood vessels were grossly normal. The liver was approximately twice normal size, and there were numerous small, gray, tubercle-like lesions beneath the capsule and on the surface of the cut section. There were no abnormalities in the gallbladder or the pancreas. The spleen was greatly enlarged, weighing 76 grams. The surface was granular and the surface of the cut section was beefy-red. Within the adrenal glands there were numerous small gray, tubercle-like lesions. None of these measured over 2 mm in diameter. The kidneys were grossly normal. The stomach, small and large intestines were not unusual, and no ulcers were found in the mucosa. The pelvic organs appeared essentially normal.

Smears were made from the cut surfaces of the spleen, the lungs, the liver, and the lymph nodes. The smears were stained with Wright's, Giemsa, hematoxylin and eosin, and acid-fast stains. The acid-fast stains revealed no acid-fast organisms in any of the lesions. However, all stains showed numerous encapsulated organism identified as *Histoplasma capsulatum*. These organisms were within the cystoplasm of large reticulo-endothelial cells (Fig. 1).

The needle biopsy from the liver revealed, on microscopic examination, numerous encapsulated organisms identified as *Histoplasma capsulatum* in the Kupffer cells of the sinusoids. Although the patient expired before tissue sections could be prepared, this positive finding indicates the applicability of this method to the diagnosis of this disease.

Stained sections for microscopic study of the various organs showed atelectasis of the lungs, with many reticulo-endothelial cells within the alveolar spaces and the alveolar walls. A large percentage of these cells were distended with *Histoplasma* organisms, many cells containing great numbers of these organisms and others containing only a few. The lumina of the small and large bronchi showed many reticulo-endothelial cells containing *Histoplasma* organisms. Sections of the hilar and mediastinal lymph nodes showed extensive

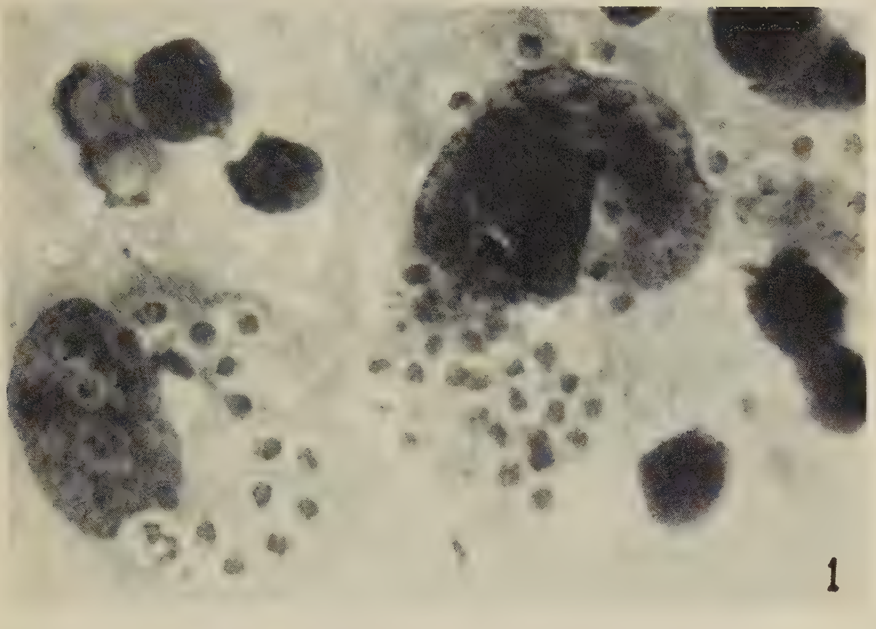

Fig. 1. Parasitized macrophage cells of the liver showing the characteristic oval bodies of *H. capsalatum.* 970 ×.

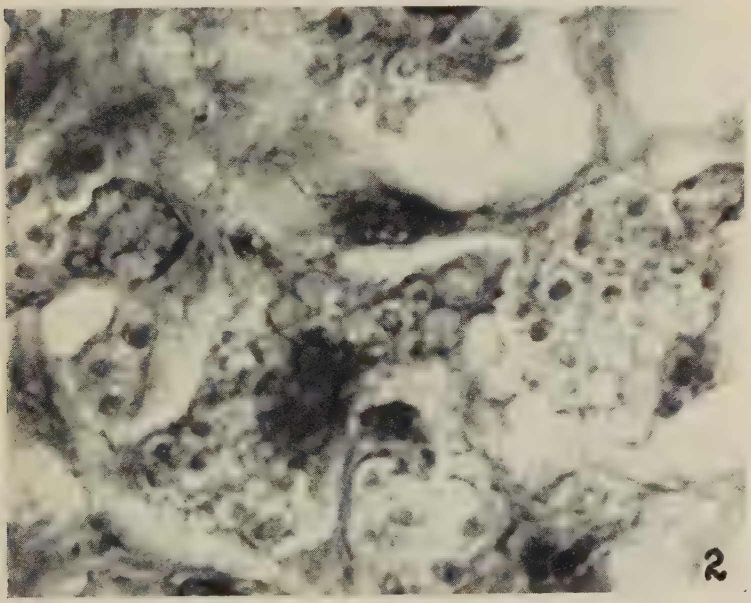

Fig. 2. Extensive dissemination of *H. capsulatum* in the reticulo-endothelial cells of the thymus. 970 ×.

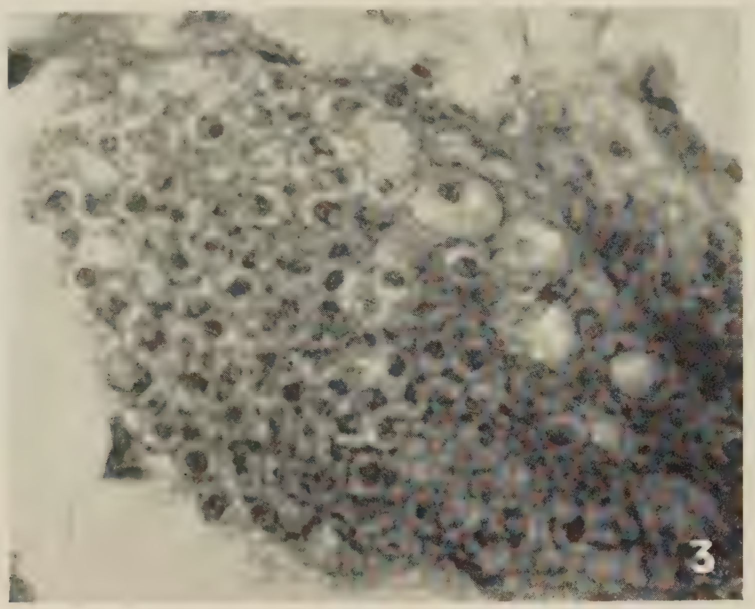

Fig. 3. Section of the mediastinal lymph node with marked *Histoplasma* involvement. 970 ×.

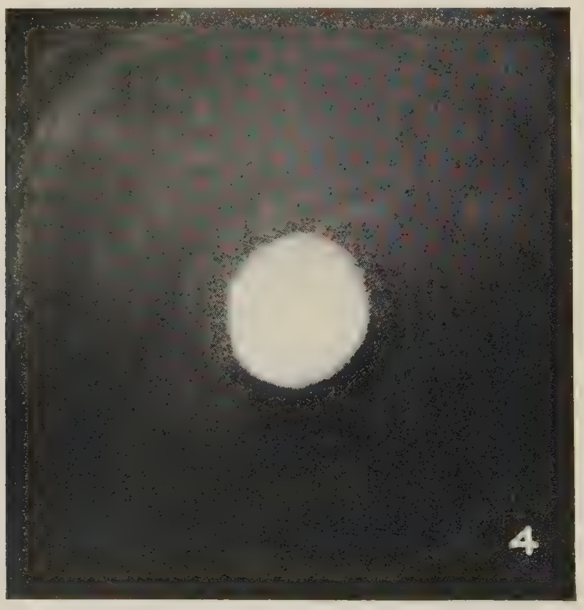

Fig 4. White, cottony colony after 14 days on Sabouraud's conservation agar at 20° C.

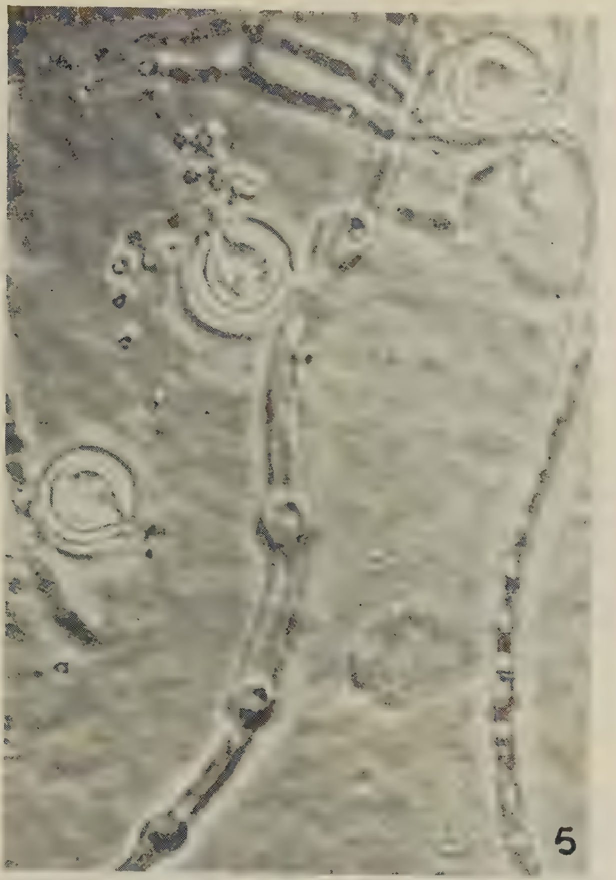

Fig. 5. Mycelial growth on Sabouraud's conservation agar incubated at 20° C. Branching, septate hyphae and racquet mycelium are both evident plus the smooth-walled spores which may be of round or pyriform shape. Phase microscopy; 430 ×.

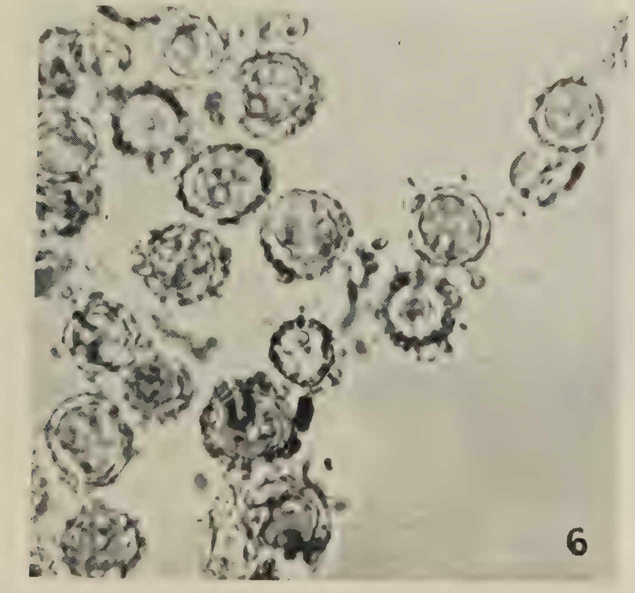

Fig. 6. The characteristic thick-walled spiny chlamydospores from a 21 day old colony on Sabouraud's conservation medium.

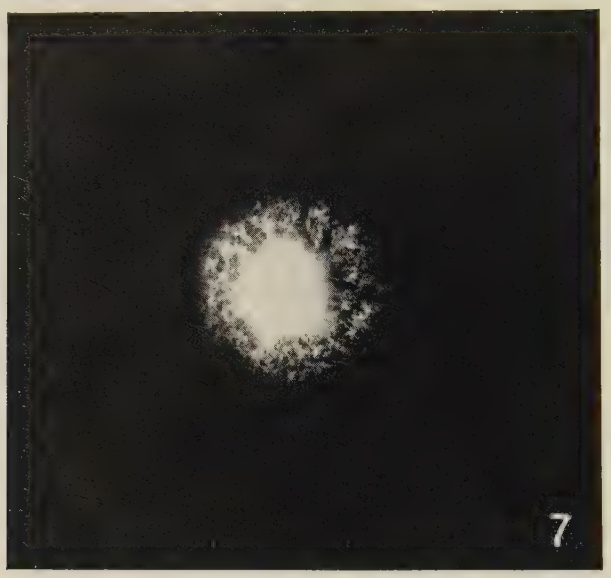

Fig. 7. *H. capsulatum* on Sabouraud's conservation agar after 14 days of incubation at 37° C

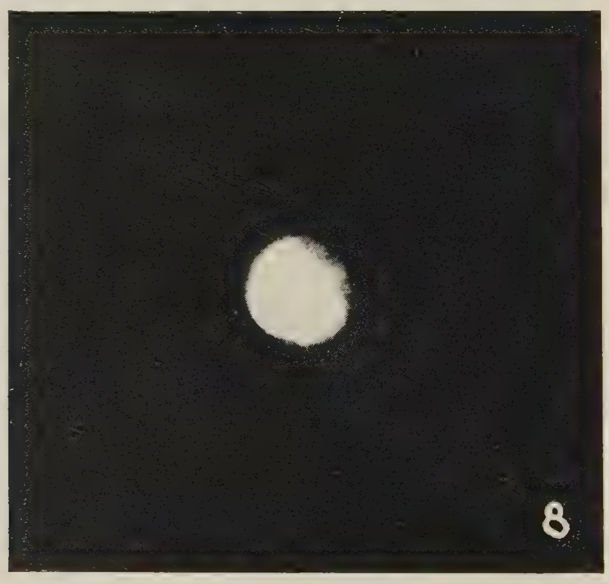

Fig. 8. Mycelial growth on Sabouraud's glucose agar after 14 days of incubation at 20° C.

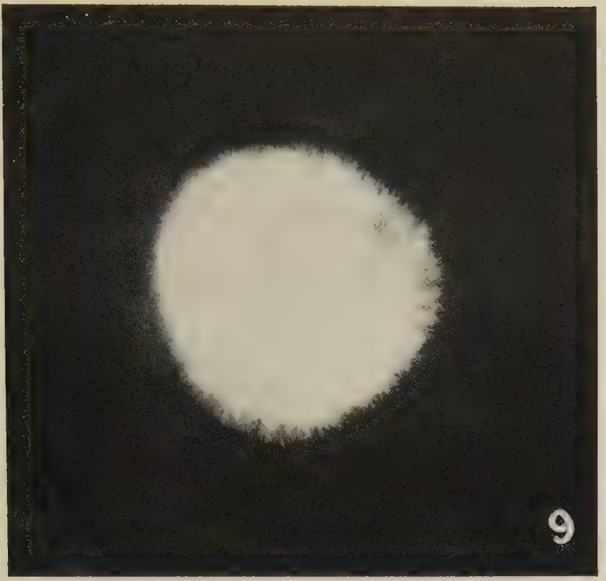

Fig. 9. Same as Fig. 8 except that incubation was at 37° C. Fig. 8 and 9 are equally enlarged. Note that the difference in colonial size between cultures incubated at 20° C and 37° C is much more pronounced on Sabouraud's glucose agar than on the conservation medium.

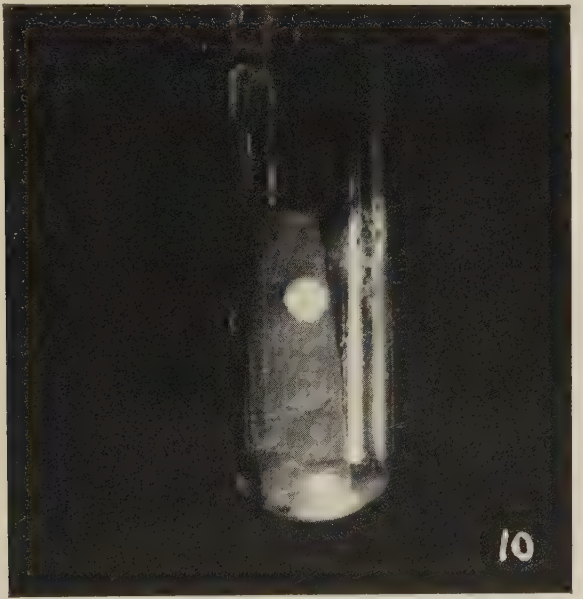

Fig. 10. *H. capsulatum* on a carrot plug after 10 days of incubation at 37° C.

caeseation of these nodes with a granulomatous reaction and numerous reticulo-endothelial cells filled with *Histoplasma* organisms. Sections from the thymus showed a marked reticulo-endothelial proliferation and the reticulo-endothelial cells were extensively parasitized by the organisms (Fig. 2 and 3).

In the liver there was a tremendous reticulo-endothelial proliferation within the sinusoids, and great numbers of these reticulo-endothelial cells were distended with organisms. Occasional liver cells also contained the organisms. Small groups of organisms were seen in reticulo-endothelial cells and phagocytes within the pancreas.

Sections of the adrenal glands showed extensive destruction with caseation, and numerous organisms in reticulo-endothelial cells. Some of these organisms were seen in the cortical adrenal cells.

Sections of the spleen showed extensive reticulo-endothelial proliferation with large numbers of organisms within the reticulo-endothelial cells.

No organisms were found in sections of the gastro-intestinal tract.

The kidneys showed small, rare foci containing reticulo-endothelial cells and *Histoplasma* organisms.

The autopsy diagnosis was: Acute disseminated histoplasmosis, with extensive involvement of lungs, thymus, mediastinal and retroperitoneal nodes, liver, adrenals and spleen, with lesser involvement in the kidneys.

Cultural Study

The organisms of *H. capsulatum* were isolated directly by inoculating macerated portions of the liver on Sabouraud's medium and on blood agar and incubating them at 20° C and 37° C, respectively. On Sabouraud's medium a luxuriant growth of the mycelial phase with tuberculate chlamdospores was produced after five weeks. On blood agar the organism remained in the yeast phase, but large colonies were produced.

After the liver had been cultured a section of it was emulsified in physiological saline and inoculated intraperitoneally into six Swiss albino mice. Three weeks later one of the females of this group gave birth to a litter of eight. These newborn mice were sacrificed and autopsied, but no pathological changes could be found and *H. capsulatum* was not recovered.

Four weeks after inoculation all six mice were sacrificed. Five of the mice showed small yellowish, glistening lesions on the mesentery, omentum, and liver; the last animal showed no macroscopic pathological changes. Impression smears made from these lesions revealed the characteristic yeast-like bodies of *H. capsulatum*.

Sabouraud's conservation medium and Sabouraud's glucose agar were inoculated with material from one of the lesions and incubated at 20° C. After 14 days of incubation a pure culture of the fungus was found, appearing as a white, cottony, aerial mycelium.

On Sabouraud's conservation medium at 20° C incubation the colony was white, cottony, with a woolly margin and capitate contour (Fig. 4). Microscopically, this culture showed branching, septate hyphae, occasional racquet mycelium, and round to pyriform smooth spores (Fig. 5). The characteristic large, thick-walled, tuberculate chlamdospores were easily identifiable after 21 days incubation (Fig. 6). The enclosed bodies in the chlamydospores, which are demonstrable in Fig. 6, are not spores but fat droplets as has been shown by HAUSMANN and SCHENKEN (1934), DEMONBREUN (1934), CIFERRI and REDAELLI (1934), and others. The fact that these bodies are not spores supports the claim made by CONANT (1941) that *H. capsulatum* should be considered a member of the Fungi Imperfecti.

When incabated at 37° C for 14 days, the culture on Sabourand's conservation medium showed essentially the same colonial characteristics as the one at 20° C, except that the edge of the colony was somewhat rougher and a crater was evident in the center of the colony (Fig. 7).

The colonies found on Sabouraud's glucose agar after 14 days incubation at 20° C and 37° C resembled those described from the conservation medium, except for the element of size; the ones incubated at 37° C were much larger than those incubated at 20° C (Fig. 8 and 9).

A study was made of the biochemical characteristics of this culture, the inoculated media being observed after 10 and again after 30 days of incubation at the indicated temperatures.

Gelatin incubated at 20° C showed sparse growth and no liquefaction.

Litmus milk incubated at 20° C and 37° C became alkaline at both temperatures but showed no coagulation or digestion.

A series of sugar and alcohols were prepared in the following medium:

Proteose peptone 3	2.0 %
Beef extract	0.3 %
NaCl.	0.5 %
Sugar or alcohol	0.5 %

One ml of 1.6 % alcoholic solution of brom thymol blue per liter.
Adjust to pH 7.0.

The cultures were incubated at 20° C and 37° C. No gas nor acid was produced in media containing glucose, sucrose, lactose, maltose, mannose, rhamnose, trehalose, dulcitol, sorbitol, mannitol, and xylose.

A carrot plug inoculated with these organisms incubated at 20° C showed only feeble growth after 10 days of incubation but the carrot plug culture incubated at 37° C showed good growth after the same period of incubation (Fig. 10). Only mycelial growth was found and the colonial morphology was indistinguishable from that found on Sabouraud's conservation agar.

Potato plugs inoculated with this culture also showed poor growth at 20° C but very good growth at 37° C. The colony was white and cottony and showed deep radial furrows. Microscopically, only mycelial growth was found, showing small, round to pyriform, smooth conidia.

Summary

The clinical history of a fatal case of acute disseminated Histoplasmosis in a 3-month-old infant is described. A liver biopsy performed ante mortem revealed numerous encapsulated organisms which were identified at H. *capsulatum*. This suggests the applicability of this technique as a diagnostic tool. An autopsy showed extensive involvement, the organisms being found in the lungs, thymus, mediastinal and retroperitoneal nodes, liver, adrenals, spleen, and kidneys.

A portion of emulsified liver was inoculated into Swiss mice and *H. capsulatum* was recovered from the mouse tissues 4 weeks after inoculation.

A study was made of the cultural characteristic of this culture and it conformed to those previously described for other strains. A study of its fermentation properties disclosed no acid or gas production in media containing glucose, sucrose, lactose, maltose, mannose, rhamnose, trehalose, dulcitol, sorbitol, mannitol, and xylose.

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Two new yeasts from Hawaiian fruit flies[1]

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The authors (1950) published information regarding the identity and ability of yeasts from Hawaiian fruit flies to produce riboflavin. Two of the yeasts are new and are described in the present paper. The methods employed for determining the morphological characters are those developed by Stelling-Dekker (1931), Lodder (1934) and Diddens and Lodder (1942). The techniques recommended by Wickerham (1946) and Wickerham and Burton (1948) were used in order to ascertain the biochemical characters of the yeasts. One of the new yeasts belongs to the *Candida krusei* series, the other yeast is a *Rhodotorula* which has some of the characters possessed by *R. sanniei*.

Candida sorbosa *n. sp.* This isolate is from the feces of *Drosophila crucigera*, collected near Poamoho Oahu. Of the sugars employed by Diddens and Lodder, this yeast ferments and assimilates only glucose. These characters are shared by *C. krusei* and *C. monosa*. This new form can assimilate 1-sorbose, while *C. krusei* and *C. monosa* cannot. The latter yeast is able to assimilate d-xylose, but *C. krusei* and *C. sorbosa* are unable to use this sugar. In our experiments, using Wickerham's synthetic medium and conditioning the yeasts in a vitamin free substrate, we found that *C. krusei* and *C. sorbosa* do not require added vitamins, while *C. monosa* needs added vitamins for growth. However, Burkholder (1943) reported that N.R.R.L. Strain Y 306 of *C. krusei* required biotin and grew better with some added thiamin. The results we obtained from the cultures collected from fruit flies are indicated in Table I.

Table I. Candida fermenting glucose only, from Hawaiian fruit flies, their need for added vitamins.

Yeasts		Growth without Added Vitamins
C. krusei	Y 1736	+
C. krusei	Y 1743	+
C. krusei	Y 1733	+
C. krusei	Y 1798	+
C. monosa	Y 1735	—
C. monosa	Y 1734	—
C. sorbosa n. sp.	Y 1732	+

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[1] Supported in part by a grant-in-aid from the Graduate School, Illinois Institute of Technology.

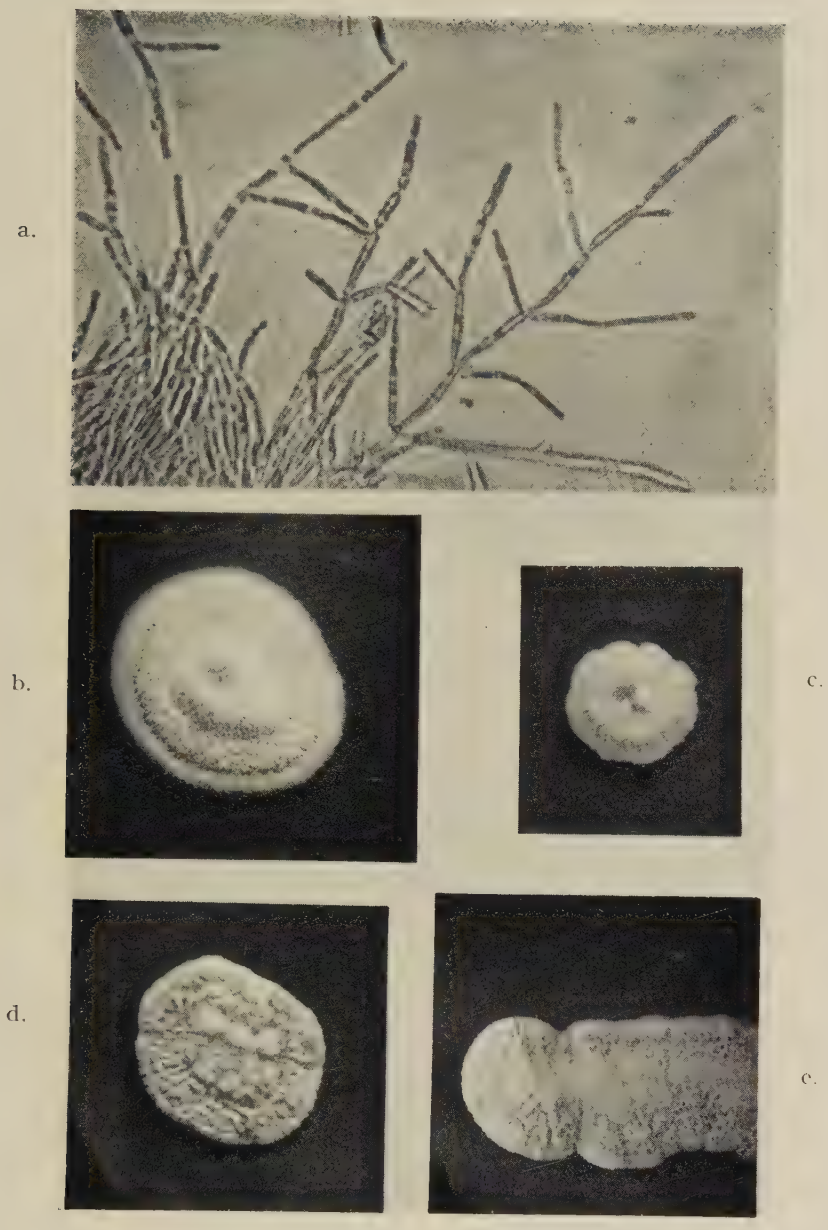

Fig. 1 a. Pseudomycelium of *Candida sorbosa* nov. sp. X450; b. Colony of *C. krusei* X3.2; c. Colony *C. monosa* X3.2; d. Colony of *C. sorbosa* nov. sp. X3.2; e. Agar streak of *C. sorbosa,* nov. sp. X3.2

In order to determine the morphological characters, the three closely related *Candida* were streaked upon WICKERHAM's synthetic fungus agar (WICKERHAM and BURTON, 1948). For the mycelial development, a small area of each streak was covered by a sterile 7/8 inch coverglass as suggested by DALMAU (1929) and WICKERHAM and RETTGER (1939). The petri plates were incubated at 25° C. for 7 days. Under these conditions our strains of *C. krusei* produced a true mycelium, while *C. monosa* and *C. sorbosa* (Fig. 1a) formed a pseudomycelium. Point inoculations were made on the plate for colony characteristics. The colony characters for the three species are as follow: *C. krusei* (Fig. 1b) margin essentially entire, border and lateral area white and fine spiny to fine woolly, central region decidedly raised, covered with white, cottony, felt-like tufts; *C. monosa*, (Fig. 1c) margin irregular, surface slightly convex with flattened central area and generally smooth; *C. sorbosa*, (Fig. 1d) margin essentially entire, lateral area, creamy white, covered with many small folds or elevations; central area cream colored and papillated. The streak characters are essentially the same as the colony characters (Fig. 1e).

In wort broth a pellicle is formed by *C. sorbosa* and the cells in the liquid are for the most part individual cells; however, some clusters of a few cells are present. The cells are oval to ellipsoidal, with measurements 8.4 μ avg (7.2 to 11.4) by 3.9 μ (2.8 to 5.6). The relationships of these 3 species are tabulated in Table II.

Table II. Characteristics of 3 species of Candida

Character	*C. krusei*	*C. monosa*	*C. sorbosa* nov. sp.
Mycelium	true	false	false
Colony	white woolly or spiny with a cottony center, 10–12 mm. diameter after 7 days at 25° C.	creamy-white colony, convex with flattened center-irregular but smooth surface. 5–6 mm. in diameter after 7 days at 25° C.	creamy-white lateral area surface discontinuously radiately wrinkled, center papillated. 8–9 mm. in diameter after 7 days at 25° C.
Fermentation	glucose only	glucose only	glucose only
Assimilation	glucose	glucose and d-xylose	glucose and l-sorbose
Growth without supplied vitamins	+	—	+

Specific diagnosis

CANDIDA SORBOSA *nov. sp.* ferments glucose only, and assimilates, glucose, l-sorbose, d-glucosoamine, ethyl alcohol, glycerine, pyruvate, lactate, and succinate; grows in absence of vitamins; forms a pseudomycelium of the *Mycocandida* type; cells elliptical, length 8.4 μ (7.2 to 11.4) long and 3.9 μ (5.6 to 2.8) wide; thin pellicle with ring and flocculent sediment in WICKER-

HAM's or wort broth; colony characteristics on WICKERHAM's synthetic agar: margin entire, lateral area creamy white covered with many discrete radiating folds or elevations, central area cream colored and papillated. Type culture is deposited at the Northern Regional Research Laboratory of the U. S. Department of Agriculture, Peoria Illinois and is recorded as number Y 1732.

CANDIDA SORBOSA *sp. n.* — Fermentationem solum glucosium inducens; glucosium, 1-sorbosium, d-glucosoaminum, alcohol ethylicum, glycerinum, pyruvatum, lactatum, succinatumque assimulans; vitaminibus carentibus crescens; pseudomycelium praesens *Mycocandida* typica; cellulae 8.4 μ (7.2—11.4 μ) longae, 3.9 μ (5.6—2.8 μ) latae; sedimentum, anulus et pellicula tenius in jure efformata; colonia margine integro, parte laterali cremeo-alba, e plicis vel elevationibus discretis radiantibus tecta, centro cremeo-tincto, papillato.

RHODOTORULA PILIMANAE *nov. sp.* — One isolate from a living larva of *Drosophila pilimanae* collected near Tautalus, Oahu, is a previously undescribed member of the genus *Rhodotorula.* Carotinoid pigment is produced, no ascospores are formed and none of the sugars are fermented. The cells are nearly spherical — 4.8 μ avg (2.8 to 5.7) by 4.7 μ avg (2.8 to 5.7). Wort agar streak is red with a tinge of orange, is soft, glistening, with slight papillations, and even margins. Biochemically it cannot assimilate nitrate and lactose, but can assimilate glucose and sucrose. According to the key in LODDER (1934) this group of characters belongs to the species *Rhodotorula sanniei.* However, when compared with *R. sanniei* for further assimilation studies, many differences were noted between this new form and *R. sanniei.* The compounds which *R. sanniei* is capable of assimilating but which the new species is unable to use are maltose, cellobiose, melezitose, rhammose, and salicin. On the other hand this isolate can assimilate calcium 2-ketogluconate, while *R. sanniei* cannot use this carbohydrate. It is similar to *R. sanniei* in being unable to grow in the absence of vitamins.

RHODOTORULA PILIMANAE *nov. sp.* — Wort agar streak is red, tinged with orange, is butyrous, glistening, slightly papillated, margin entire. Cells nearly spherical, 4.8 μ, avg (2.8 to 5.7) by 4.7 μ, avg (2.8 to 5.7). No fermentation, can assimilate glucose, galactose, 1-sorbose, sucrose, calcium, 2-ketogluconate, raffinose, and ethyl alcohol. Cannot assimilate potassium nitrate, lactose, maltose, cellobiose, melezitose, rhamnose and salicin. Cannot grow in absence of added vitamins. Type culture is recorded as Y 1738 at the Northern Regional Research Laboratory, Peoria, Illinois.

RHODOTORULA PILIMANAE *sp. n.* — Linea in agaro maltoso aurantio-rubro, butyrosa, nitens, leniter papillata, margine integro; cellulae fere sphaericae, medio 4.8 μ (2.8—5.7 μ) × 4.7 μ (2.8—5.7 μ); fermentatio abest; glucosium, galactosium, 1-sorbosium, sucrosium, calcium 2-ketogluconatum, raffinosium et alcohol ethylicum, sed non potassium nitratum, lactosium, maltosium, cellobiosium, melezitosium, rhamnosium nec salicinum assimulantur; vitaminibus additis carentibus crescere not potest.

Acknowledgments

The authors wish to acknowledge thanks to Dr. LYNFERD WICKERHAM of Northern Regional Research Laboratory for checking our sugar assimilations and determining the assimilation reactions for a variety of carbohydrates. We express appreciation to Dr. M. A. COUNTRYMAN of the Institute for aid in the photographic work, to Dr. G. B. MAINLAND for collecting the microbes from the wild fruit flies, to Difco Company for the experimental WICKERHAM's media, and to Miss EDITH K. CASH, U.S.D.A., Bureau of Plant Industry, Beltsville, Maryland for the Latin diagnoses.

Summary

One yeast isolate from the feces of the fruit fly *D. crucigera* is new and is described as *Candida sorbosa nov. sp.* A new species of *Rhodotorula* from the living larva of *Drosophila pilimanae* is described as *Rhodotorula pilimanae.*

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Osservazioni sui corpi ifici di Delacroixia coronata (Cost.) Sacc. et P. Syd. in coltura

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received Sept. 23th 1950

Nel 1925, G. W. MARTIN notava, nella morfologia di *Delacroixia coronata* (Cost.) Sacc. e P. Syd., particolari aspetti del micelio vegetativo, che questo A. (MARTIN, 1925) così riassume:

> ,,As the hyphae grow, the older parts tend to become emptied of protoplasm, and cut-off by septa from the protoplasm-filled tip. Frequently a septum appears before the protoplasm is all withdrawn, and in this way numerous segments are isolated, filled with protoplasm, with emptied and shrunken hyphal portions on either side. If food is abundant these segments may send out lateral branches almost at once. In old and exhausted or dried cultures they tend to remain dormant and to accumulate, assuming various enlarged shapes, and forming hyphal bodies entirely homologous with the well-known structures to which that name is applied, formed by the Entomophthoraceae attacking insects.'' [1])

In *D. coronata* dunque l'allungamento delle ife non è di solito seguito dal completo spostamento del plasma in avanti, sicchè nel decorso delle ife stesse si alternano tratti di plasma ed intervalli di pareti vuote.

Non sempre però un setto ,,appare prima che il protoplasma sia del tutto ritirato'' (MARTIN, loc. cit.) dal micelio vecchio in decadimento. Anzi l'apparire del setto è talora tardivo. E d'altra parte, lo stesso apparire precocemente del setto già sarebbe degno di nota; essendo, come è noto, in questi funghi, i setti legati nel loro differenziarsi alla separazione di corpi ifici od a vecchiaia pregressa — anche se locale — del micelio, ed essendo quindi completi.

Il plasma così frammentato emette di solito immediatamente ife laterali o subapicali di diametro nettamente inferiore a quello dell'ifa da cui provengono; talvolta però, interrompendo il proprio sviluppo, mostra alcuni peculiari caratteri morfologici, rilevabili all'osservazione microscopica.

E su alcuni di questi caratteri credo opportuno soffermarmi brevemente nel corso di questa nota.

Il metodo seguito per compiere tali osservazioni, per le quali sembrava necessario lasciare indisturbata la posizione delle ife e degli elementi che le costituivano, fu il seguente.

Colture del fungo su vetrino copri-oggetto in mezzo agarizzato di Couch (1939) (maltosio gr. 3, peptone gr. 4, H_2O litri 1, agar gr. 20) vennero fissate ,,in toto'' in liquido di Schaudinn (più acido acetico), lavate in soluzione iodo-iodurata alcoolica, in tiosolfato sodico ed infine in acqua. Non si potettero adoperare colorazioni del tipo FEULGEN—DELAMATER (1948a, 1948b) per le conseguenze dello HCl a caldo (60° C) sul sottile agar. Ci si attenne pertanto principalmente a: Ematossilina Heidenhain ed Ematossilina Delafield. Il vetrino fu poi sottoposto a contrasto (safranina) e disidratazione per l'inclusione in balsamo o, più semplicemente, dopo accurato allontanamento dello allume ferrico-ammoniacale, fu capovolto sul portaoggetti ove si era deposta una larga goccia di glicerina diluita in acqua. L'osservazione venne effettuata il giorno dopo, anche in questo ultimo caso.

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[1]) Non so invece se in qualche modo sono da ravvicinare alle mie osservazioni quelle di Kevorkian (1937):

,,However, the appearance in the Cuban material of certain thick-walled structures closely resembling zygospores, needs further critical study to determine their true nature.'' Noterò al riguardo che più volte notai, specie in caso di competizione fra due o più colonie del fungo, aspetti morfologici del micelo, in particolare nel suo margine avanzante, che lasciavano sospettare possibili fatti sessuali, ma che tuttavia non furono mai visti froseguire.

A circa 23° C i particolari aspetti del micelio che si riassumeranno in seguito apparvero entro 5 giorni. Entro 9 giorni detti corpi erano tanto evidenti da sporgere in un alone di acqua di condensazione sul sottile strato di agar in mezzo al vecchio micelio in lisigeni. Allora, osservando più attentamente, si notava che qua e là il plasma stesso si rivestiva internamente all'ifa di un secondo, spesso (circa μ 1) tegumento, nettamente visibile negli esemplari in plasmolisi, perchè dotato di sufficiente rigidità e pertanto non contraentesi insieme al plasma. I corpi così determinatisi, di forma allungata od ovale o perfettamente sferica, sono anche facilmente distinguibili per il plasma densissimo, che fissa tenacemente l'ematossilina dei coloranti.

Si è detto che tali corpi possono rivegetare, se così può dirsi, immediatamente, emettendo, contemporaneamente o quasi, fino a 10 ife. Tale divisione avviene in modo vario, ma che sembra prevalentemente riconducibile a due schemi principali. Nelle forme allungate infatti l'apparire del setto può essere preceduto dalla formazione di un grosso vacuolo centrale che divide il plasma e lo spinge verso le due estremità del corpo che si descrive (fig. 1). Così, lateralmente a questo vacuolo, si formano due setti, che, in forme particolarmente allungate, possono essere seguiti da ulteriori divisioni prima dell'emissione di nuove ife (fig. 2). Specie se il corpo è equidiametrale, invece, il citoplasma ed i nuclei si addensano al centro della cellula; e, se il setto si differenzia, il plasma, a seconda della sua quantità, si sposta quasi tutto in una sola delle due cellule figlie (mentre nell'altra si differenzia un grosso vacuolo) o si suddivide fra di esse.

Nei corpi, in particolare, superanti periodi di apparente inattività, si è cercato di seguire il comportamento dei nuclei. Nonostante si siano più volte viste anastomosi — comuni nel fungo — adiacenti ai punti di differenziazione di essi corpi, non è stato possibile osservare fenomeni di interesse nei nuclei, nè sensibile riduzione del loro numero (vedansi al proposito: CAVARA (1899) e VUILLEMIN (1900)). Talora solamente alcuni di essi apparvero più grandi e meno definiti degli altri nel contorno. I nuclei delle ife in vegetazione sono poi addossati alle pareti, per lo più; mentre nel nostro caso sembrano piuttosto sparsi al centro del citoplasma (fig. 3).

Sono note nelle *Entomophthoraceae* forme asessuate ed a volte forse apogame, distinte come: azigospore, partenospore, ecc. Per lo meno in alcuni casi, tali denominazioni sembrano ancora, come cinquanta anni fa (VUILLEMIN, 1900), appoggiate ,,à des analogies de forme et de fonction''. Sembra pertanto più immediato il ravvicinamento dei corpi che ci determinano nel modo descritto a clamidospore, seguendo in questo quanto parrebbe implicitamente ammesso anche di recente da SMITH (1938) e da LANGERON (1945).

Summary

The author follows the formation, the resting features and the further development of the peculiar hyphal bodies of *Delacroixia coronata*. By the microscopical examination of colonies grown in Couch medium on cover-slips,

he distinguishes various schemes of development and septation in the hyphal bodies recorded above and, after having mentioned the uncertainty of some other denominations (azygospores, parthenospores), he states that they are to be considered chlamidospores in the sense of LANGERON.

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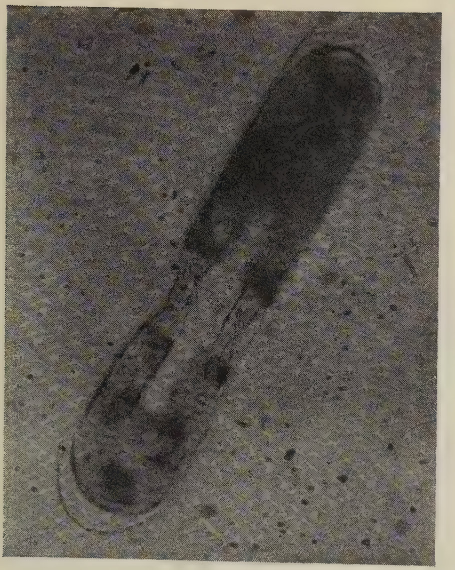

Fig. 1

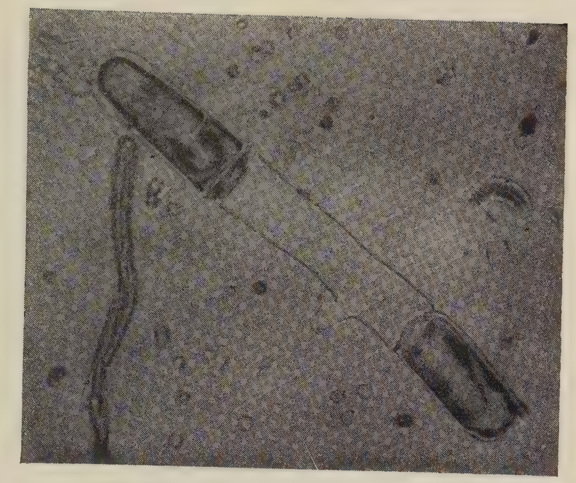

Fig. 2

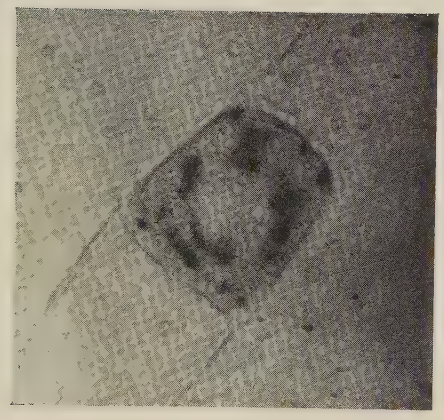

Fig. 3

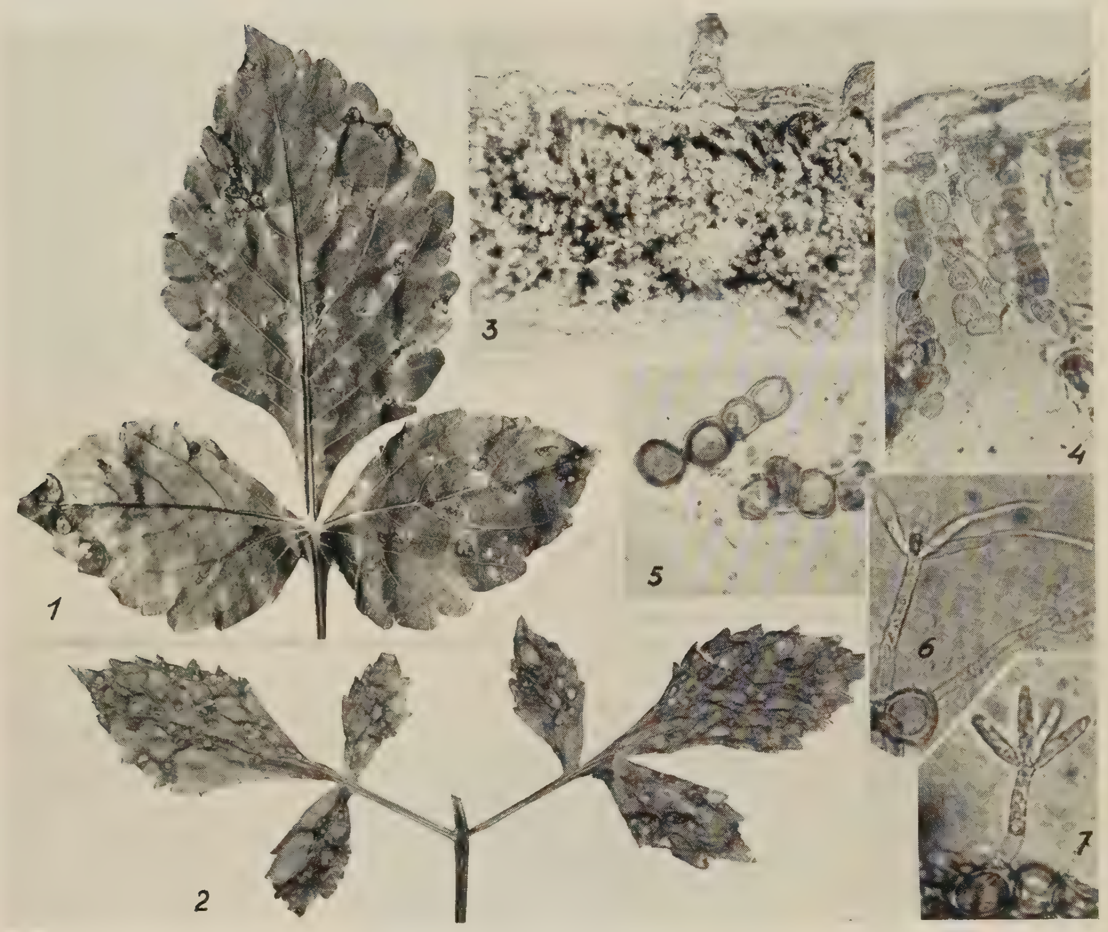

Fig. 1. Foglioline di Dahlia con macchie iniziali causate da *Entyloma Dahliae* Syd.
Fig. 2. Idem. con macchie vecchie disseccate.
Fig. 3. Sez. trasv. di mesofillo infarcito di clamidospore di *E. Dahliae* (ingr. 160).
Fig. 4. Catenule di clamidospore (ingr. 400).
Fig. 5. Clamidospore mature (ingr. 800).
Fig. 7. Clamidospora germinante, con promicelio e sporidi (ingr. 1000).
Fig. 6. Idem. e formazione di sporidi secondari (ingr. 1000).

Prima segnalazione in Italia della *Entyloma Dahliae* Syd. parassita delle dalie

di

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(7.3.1951)

Al principio dello scorso settembre il tecnico del nostro Istituto, sig. PINOGGI G., raccoglieva in un giardino di Pollone (prov. di Vercelli) numerose foglie di Dalia di una var. ,,a fiore di cactus'' (*Dahlia Juarezi* hort.) che presentavano macchie assai evidenti e numerose. Un rapido esame del materiale mi consentì di stabilire che si trattava di una infezione d'*Entyloma Dahliae* Syd., e poiché questa specie finora non é stata segnalata in Italia, ritengo opportuno descrivere brevemente la malattia ed il suo agente.

La malattia é caratterizzata dalla presenza sulle foglie di macchie inizialmente circolari, isolate, indeterminate, le quali a causa del loro colore verde chiaro contrastano in maniera assai appariscente col verde scuro delle porzioni sane del lembo (fig. 1). In questa fase esse hanno un diam. di 2—5 mm., ma poi rapidamente ingrandiscono assumendo, nello stesso tempo un contorno via via più definito ed un colore grigio-verdastro, e talvolta confluiscono, se molto numerose e ravvicinate, formando macchie irregolarmente poligonali. Le macchie isolate possono raggiungere il diam. di circa 1 cm. o poco più; quelle composte sono assai più grandi restando tuttavia limitate nella loro espansione dalle nervature primarie. Infine le macchie disseccano e diventano cenerognole nella parte centrale, che nel frattempo é andata sempre più assotigliandosi, mentre la parte periferica viene a costituire un'orlatura di colore bruno (fig. 2). Quando l'infezione ha luogo precocemente, su foglie giovani, le macchie danno origine col tempo a distorsioni e bollosità, talvolta abbastanza accentuate, del lembo e ciò — come in altre malattie fogliari — per il fatto che non essendo in grado le porzioni infette di partecipare all'accrescimento in superfice del lembo, esse vengono sopravvanzate dalle parti sane, con la conseguente formazione, specialmente quando la macchie sono numerose, di concavità, di convessità e distorsioni di vario genere fino alla totale deformazione della lamina.

Nel caso da me studiato la malattia colpiva solo le foglie più basse, apparentemente senza recare grave turbamento alle piante che, infatti, fiorirono regolarmente. Tuttavia, come riferiscono altri Autori, in casi gravi tutte le foglie vengono attaccate, anche nei piccioli sui quali dà luogo a macchie lineari o ovali-allungate, brune.

In corrispondenza delle macchie il mesofillo è infarcito di clamidospore (fig. 3), mentre un micelio ialino, sottile, serpeggia tra le cellule, in parte deformate e plasmolizzate, dei parenchimi assimilatore e lacunoso. Le clamidospore, riunite in numero fino 10—12 in brevi catenule, (fig. 4), sono da prima ialine, poi con l'età diventano gialle ed infine bruno-scure. A maturità esse sono globose o irregolarmente poliedriche (in seguito a reciproca compressione), di diam. variabile da 12,5 a 16 micr. e dotate di un episporio abbastanza grosso (1,5—2,5 micr.) fortemente rinfrangente, costituito da tre strati dei quali l'esterno più spesso e brunastro, il mediano più sottile e quasi ialino, l'interno sottilissimo e ialino (fig. 5). Le clamidospore germinano già *in loco* producendo un promicelio cilindrico (lung. 20—35 micr.) che all'apice dà origine ad un verticillo di 4—6 sporidi ialini, fusiformi-allungati, diritti o leggermente arcuati, lunghi fino a 15—16 micr. (fig. 7). Gli sporidi a loro volta possono germinare *in situ* dando origine a sporidi secondari filiformi od aciculari lunghi 45 (media 60)—75 mcr. e larghi 2 micr. (fig. 6); in goccia pendente (acqua)essi sono più piccoli (media 25 × 1,5 micr.). La formazione degli sporidi si manifesta sulle macchie con la comparsa di un efflorescenza bianca, leggera ed assai fugace.

I suddetti caratteri del parassita concordano con quelli di *Entyloma Dahliae* Syd. (Ustilaginales, Tilletiaceae) segnalata dal SYDOW (Ann. Mycol. X, 1912, p. 36) su foglie di *Dahlia variabilis* a Harden Heights, Natal, nel 1911 e descritta come specie nuova e non già, come taluni Autori ritengono, derivata dalla scissione della specie collettizia *E. Calendulae* (Oud.) De Bary.

La prima segnalazione del fungo in Europa (Belgio) risale al 1918 ed é dovuta allo STERNON (Une maladie nouvelle du Dahlia, Bruxelles, 1918) il quale però la ritenne una forma biologica di *E. Calendulae* proponendo per essa il nome di *E. Calendulae* f. sp. *dahliae*. Il CIFERRI (Prima contribuz. allo studio degli Ustilaginales. Bull. Soc. Bot. Ital. 1924 p. 48) ne fece una specie con la n. comb. *E. Dahliae*, in quanto a questo A. era sfuggita, come egli stesso riconobbe successivamente (Seconda contrib. ecc. Atti Ist. Bot. Pavia, Ser. III, vol. I, p. 92), la precedente diagnosi sydowiana. Nel 1924 GONZALEZ FRAGOSO (Ustil. de la Fl. Españ. ecc. Boll. Real. Soc. Esp. Hist. Nat. XXIV, 1924, p. 123) riportò un *E. Dahliae* su foglie di *Dahlia variabilis* raccolte a Oviedo (Spagna) da UNAMUNO nel 1922, dichiarando che questa specie é sicuramente differente da quella sydowiana; a sua volta CIFERRI (Terza contrib. ecc. Atti Ist. Bot. Pavia, Ser. III, vol. II, 1925, p. 9) per ovviare alla omonimia propose per essa il binomio *E. Unamunoi*. Infine lo stesso CIFERRI (Quarta contrib. ecc., Ann. Mycol. XXVI, 1928, p. 58) assimilò *E. Unamunoi* a *E. Dahliae* Syd., convinto trattarsi della medesima specie.

In breve la sinonimia si presenta come segue:

Entyloma Dahliae Sydow (1911);
,, *Calendulae* f. sp. *dahliae* Sternon (1918);
,, *Dahliae* Ciferri (1924);
,, *Dahliae* Unamuno (Gonzales Fragoso, 1922);
,, *Unamunoi* Ciferri (1925).

L'autonomia specifica di *E. Dahliae* é stata recentemente messa in dubbio da SAVILE (Canad. J. Res. Sect. C. XXV, 3, pp. 105—120, 1947; cit. e Rew. Appl. Myc. XXVII, 1949, p. 101) il quale in uno studio comparativo delle specie di *Entyloma* viventi sulle composite nell'America settentrionale, la considera come probabilmente appartenente al complesso ,,*Calendulae-polysporum*'' di cui sono capostipiti *E. Calendulae* (Oud.) De Bary, specie europea vivente su *Calendula*, diffusa ovunque compresa l'Italia, ed. *E. polysporum* (Peck.) Farlow, specie americana vivente su varie Composite e secondo l'A. assai affine alla precedente. Lo stesso SAVILE però riconosce che in assenza di ricerche appropriate, é d'uopo mantenere la specie sydowiana *E. Dahliae* come entità indipendente.

Dopo la sua comparsa in Europa, e precisamente nel Belgio (Sternon l.c.) il fungo si é rapidamente diffuso nel continente. Tralascio per brevità le indicazioni bibliografiche relative alle successive tappe di quest'espansione (indicazioni che sarà facile trovare consultando le annate della Rew. of Appl. Myc. sulla scorta degli autori citati) le quali, in ordine cronologico di segnalazione, sono le seguenti: 1918 Olanda (VAN POETEREN, 1922), 1922 Spagna (GONZALEZ FRAGOSO, l.c.), 1923 Francia (ARNAUD, 1925), 1924 Germania (PAPE, Gartenwelt, XXX, 40, pp. 632—634; 42, pp. 666—667, 1926), 1925 Polonia (SIEMASZKO, 1933), 1926 Moravia (Cecoslovacchia) (BAUDYŠ 1926), 1927 Inghilterra (PETHYBRIDGE, Gard. Chron. LXXXV, 2186, pp. 393—394, 1928), 1929 Svizzera (MAYOR), 1930 Austria (FISCHER, 1931), 1936 Romania

(Savulescu, Aronescu, Sandu-Ville e Alexandri, Storea fitosanitaria in Romania in anul 1935—1936, Pubbl. Inst. Cerc. Agron. României, 38, pp. 70, 1937), 1938 Svezia (Ingelström).

Fuori d'Europa la sua presenza é stata segnalate oltreché nel Natal (Africa merid.) dal Sydow (1911 l.c.), a Sumatra (Isole della Sonda), 1924 (Palm, Phytopath. XXII, 1932, pp. 868—869); nel Guatemala, 1927 (Palm, 1932, l.c.); nel Transvaal (Africa merid.), 1932 (Pole Evans); nelloArcipelago di Madera, 1936 (Viennot-Bourgin, Ann. Ec. Agric. Grignon, Ser. 3, I, 1938—39, pp. 69—169); a Ceylon, 1936 (Park, 1937); nell'Argentina, 1939 (Marchionatto); nella Nuova Zelanda, 1939 (Brien); in Brasile, 1939 (Bitancourt, 1940); nell'Oregon (Stati Uniti), 1940 (Mc Whorter); nello Uganda (Africa equat. orient.), 1940 (Ainsworth, 1941); nella Isola Mauritius (Oceano Indiano, Arcipel. delle Mascareigne), 1940 (Wiehe, 1941); nel Venezuela, 1940 (Muller, 1941); in Palestina, 1944 (Rayss e Zwirn); nella Nuova Galles del Sud (Australia sud-orient.), 1946 (Anominó, Agr. Gaz. U.S.W. 1947); nel Madagascar, 1946 (Bouriquet); in India, 1947 (Ramakrishnan T. S. e Ramakrishnan K.); nell'Angola (Africa occ. subequat.), 1948 (Noronha 1949). Nonostante l'ampiezza della diffusione di *E. Dahliae* in Europa (come si vede dall'elencazione su riportata essa é ormai presente in tutti i paesi confinanti col nostro) la specie non é stata segnalata ancora in Italia. Infatti essa non é menzionata dal Ciferri nel volume della Fl. Crypt. It. (Pars I, Fungi, 1938) dedicata alle Ustilaginales, ove sono elencate tutte le specie segnalate sino al 1933; e neppure la si trova citata nella letteratura micologica e fitopatologica successiva.

Poiché da una rapida inchiesta presso i floricoltori e dai sopraluoghi effettuati personalmente risulta che la malattia é per ora circoscritta alla località della prima segnalazione, non ho avuto occasione di fare ricerche né sulle relazioni tra ospite e parassita né sul comportamento di quest'ultimo nelle condizioni di clima, terreno e metodi di coltivazione particolari alle culture di Dalie nella nostra regione. Mi limito perciò a riassumere brevemente quanto in proposito risulta dalle osservazioni concordanti dei numerosi Autori che se se ne sono occupati.

Quanto alla recettività — premesso che *Entyloma Dahliae*, come i suoi congeneri, é specializzata su ospiti appartenenti ad un solo genere — sembra assodato che essa colpisce di preferenza le razze derivate dalla *Dahlia pinnata* Cavan. (= *D. variabilis* Desf.) e tra queste in primo luogo quelle appartenenti al gruppo delle *Dalie Cactus* (= *D. Juarezi* hort.) cosi chiamate per i grandi capolini simili ai fiori di certi Cactacee; inoltre le cosiddette „*dalie decorative*", e le „*d. a fiore d'anemone*" ed a „*fiore di peonia*". Sono pure soggette alla malattia, con grado diverso di intensità, le razze derivate dalla *D. coccinea* Cavan., come le ben note „*d. a collarino*", nonché gli ibridi della *D. coccinea* con le *D. Cactus* chiamati col nome di „*d. olandesi*" e apprezzate per i loro capolini grandissimi semplici o doppi. Più resistenti sembrano le cosiddette „*d. Pompon*" (*D. variabilis* var. *microcephala* hort.), o per lo meno talune razze di questa varietà, e le razze „*nane*" o „*lillipuziane*" (*D. pinnata* f. *nana* André = *D. lilliputiana* hort.) e. „*parigine*" (seminane), tutte derivate dalla *D. pinnata*. Immuni sembrano invece essere le *D. Merckii* Lehm., *D. excelsa* Benth., *D. imperialis* Roezl. tutte originarie, come quelle precedentemente citate, del Messico, ma poco coltivate. A tale proposito il Palm (l.c.) studiando le specie *D. coccinea* e *D. excelsa* nel loro ambiente originario e naturale (Messico e Guatemala) osservò che mentre la prima era molto colpita dal'*E. Dahliae* la seconda era immune; e ritiene tale osservazione non priva di valore in quanto *D. coccinea* é considerata come una sp. ancestrale delle Dalie coltivate. Non quindi il Natal, dove il fungo venne scoperto per la prima volta, ma dove l'ospite (*D. variabilis*), é pianta importata, ma il Messico o il Guatemala, antica patria delle Dalie coltivate, sarebbe il vero centro di irradiazione della malattia.

La malattia é favorita dall'umidità, dal l'aduggiamento, dalle semine tardive e troppo fitte e dalla deficenza di calcio del terreno. Tale deficenza predispone alla malattia anche le var. resistenti. I concimi potassici aumentano la resistenza delle piante; non cosi i superfosfati e la calciocianamide (Savulescu, Aronescu, Sandu-Ville e Alexandri 1937 l.c.). In genere la malattia non arreca gravi danni alle culture, ma in condizioni particolarmente favorevoli al patogeno e in piante giovani di razze molto recettive può provocare la defogliazione totale e anche nei casi leggeri compromettere gravemente il vigore dell'ospite (GREEN, Journ. Roy. Hort. Sc. LVII, 1922, pp. 332—339, cit. e Rew. Appl. Myc. XII, 1933, p. 26). La riduzione della capacità assimilatrice delle foglie può avere come conseguenza uno sviluppo ridotto delle radici tuberose volgarmente detti ,,tuberi'' (PAPE, 1926, l.c.).

Durante la vegetazione le infezioni si ripetono mediante gli sporidi che si formano continuamente e in grande numero sulle macchie, se il tempo si mantiene caldo e umido, e che vengono diffusi dai venti e dalle piogge; l'infezione é favorita dai medesimi fattori. E'dimostrato (VIENNOT-BOURGIN: Le charbon des feuilles de Dahlia, Biologie, Essais de traitement. J. Intern. du Dahlia, Gembloux, 1947) che il parassita può svernare nei tuberi allo stato di micelio perennante, contrariamente all'opinione di alcuni Autori, secondo cui la malattia non sarebbe trasmissibile mediante i tuberi (PAPE, 1926 l.c.); inoltre mediante le clamidospore contenute nelle foglie morte cadute e rimaste sul suolo. In primavera queste ultime germinano formando gli sporidi che danno luogo alle nuove infezioni.

Lotta. Non si conoscono metodi sicuri di lotta. Il coltivatore di Dalie deve sorvegliare attentamente le sue colture e distruggere tutte le foglie ammalate già dalle prime manifestazioni (macchie verdi chiare) e continuando durante tutto il periodo vegetativo. Sarà bene affiancare questo metodo distruttivo con alcune irrorazioni di poltiglia bordolese normale, a partire dalla primavera, ripetendole ogni 3—4 settimane, e mescolando alla poltiglia qualche adesivo. L'utilità reale di questi trattamenti é controversa: mentre in Inghilterra PETHYHRIDGE (1928 l.c.) e in Belgio (ROEKENS, Cult. en Handel, XII, 7, 1947, p. 27 cit. e Rew Appl. Myc. XVII, 1948, p. 245) ne hanno constatato l'efficacia, in Francia non sempre se ne ebbero vantaggi apprezzabili (VIENNOT-BOURGIN, 1927 l.c.). Buoni risultati sono stati ottenuti in Svizzera con irrorazioni di una soluzione di carbonato di rame al 0.3—0,4 %. Nonostante i dubbi sussistenti sui vantaggi di tali trattamenti non é possibile, allo stato attuale degli studi consigliare di meglio, essendo ancora prematuro parlare di una eventuale traduzione in pratica delle esperienze tentate da VIENNOT-BOURGIN (1927, l.c.) con l'impiego di alcuni coloranti basici come l'auramina, la crisoidina, l'orange d'acridina, la rodamina ed altri.

In autunno il coltivatore dovrà adoperare la massima diligenza nel ripulire accuratamente il terreno dai residui delle foglie morte, prima di dissotterrare i tuberi, onde evitare con la zappatura le foglie infette vengano incorporate al terreno ove le clamidospore possono restare vitali per molti anni. Infatti FISCHER (Phytopathology XXVI, 1936, p. 1118—1127), ha dimostrato che le clamidospore di *E. Dahliae* sono capaci di germinare anche a distanza di 10 anni in proporzione del 19 %, nonostante che le foglie da lui usate nelle ricerche fossero state disinfettate mediante tetracloruro di carbonio o sublimato corrosivo e successivamente conservate in erbario con naftalina. D'altro canto se il terreno non é mondo di foglie infette le clamidospore possono per adesione contaminare anche i tuberi sani i quali cosi diventano veicolo di nuove infezioni. Va da sè che nell'impianto di nuove colture occorre scegliere un terreno non infetto e tuberi provenienti da colture esenti dalla malattia.

E'buona regola far precedere la semina, che non deve essere troppo fitta, da una vangatura profonda; saranno anche utili la calcitazione del suolo e l'uso di concimi potassici in leggero eccesso sugli altri.

Summary

The author points out for the first time in Italy (Prov. of Vercelli, Piemonte) *l'Entyloma Dalhiae* Syd. a parasite on *Dahlia laurezi* hort. He revises the systematic position of the fungus, he describes again the disease and specifies the geographic distribution.

He gives indication for the teraphy.

Binomi negletti o mal noti di specie fungine nell'opera di S. Rivolta

di

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(10.6.1951)

L'opera fondamentale del Professore de Veterinaria Sebastiano Rivolta (passato alla storia quale scopritore dell'agente del farcino equino, oggi *Histoplasma capsulatum* (Riv. et Mic.) Cif. et Red.) rimane il volume ,,Dei parassiti vegetali come introduzione allo studio delle malattie parassitarie e delle alterazioni dell'alimento degli animali domestici'' (Tip. di Giulio Speirani e Figli. 521 pagg., 10 tav. in lit., con 322 figg. in nero 1873), di cui sembra rimangano oggi solo pochi esemplari in circolazione.

Giá Direttore dell'Istituto Zooiatrico di Torino, fu quindi Professore di Patologia Generale ed Anatomia Patologica dell'Istituto Zooiatrico della Universitá di Pisa, visse ed operó in un interessante periodo di transizione, per quel che riguarda le malattie degli animali domestici ad eziologia fungina. Pienamente riconosciuta tale eziologia, lo studio dei cicli biologici delle specie, imposto se non fondato da Antonio de Bary (di cui era uscita la classica opera ,,Morphologie und Physiologie der Pilze, Flechten und Myxomyceten''. Leipzig, Engelmann. I ediz. 1868; II ediz., 588 pagg., 1884), mentre da un lato incontrava fieri oppositori, dall'altro ebbe troppo fervidi aderenti, quali quell'Ernesto Hallier la cui opera fondamentale (,,Parasitologische Untersuchungen'', Engelmann, Leipzig, 1868), oggi quasi sconosciuta, ebbe allora notevole risonanza e ferventi adepti delle di lui idee. L'Hallier, ampliando a dismisura la nascente nozione o dottrina, fondata dal Tulasne, circa il pleomorfismo nei funghi, tentava di stabilire dei cicli, da lui dette ,,morfe'' per cui, partendo da banali specie fungine (generalmente muffe degli Ifomiceti o delle Mucoracee), si giungeva a forme aventi delle specifiche eziologie patogenetiche o ,,fermenti'', quali micrococchi, criptococchi, artrococchi, ,,zoococchi'', ecc. Al lato opposto erano parecchi studiosi, tra cui degli illustri, micologi del tempo, ad es. H. F. Bonorden (cfr. l',,Abhandlungen aus dem Gebiete der Mykologie'', Halle, Vol. I, 1864) che nelle connessioni metagenetiche tra forme di una stessa specie fungina vedevano solo un ,,rapporto di coabitazione''.

Il Rivolta, pur rifiutando la rigiditá degli schemi del Bonorden, tenta di attenuare le esagerazioni dell'Hallier, sebbene indulgendo, purtroppo non raramente, all'idea delle ,,morfe''.

Ma non c'interessa qui questa questione, sibbene il fatto che nel volume sono indicate o descritte o figurate numerose specie fungine, o vengono create delle combinazioni o riportati dei sinonimi di cui, a quel che pare, P. A. Saccardo non tenne conto nella redazione della sua monumentale ,,Sylloge Fungorum''. Poiché un certo numero di binomi potrebbe considerarsi come validamente pubblicati, ne diamo un'elencazione con qualche nota critica. Tralasciamo quanto concerne il genere *Cryptococcus* al quale abbiamo fatto piú dettagliato riferimento in una nota in inglese in questa stessa rivista.

pag. 57. — *Mucor sanguineus* Del Col apud Rivolta. E'il nome che il chimico DEL COL, secondo RIVOLTA, avrebbe assegnato alla sostanza colorante (?) del *Micrococcus prodigiosus*, da lui chiamato *Zoogalactina immetrofa*. Si riferisce alle famose esperienze del medico padovano V. SETTE che nel 1824 dimostró la causa microrganica del fenomeno del ,,pane'' e delle ,,ostie con sangue'', noto sin dall'antichitá, e che tanto chiasso fece in quel di Padova nel 1819. RIVOLTA riferisce la *Zoogalactina immetrofa* (*Serratia maydis* Brizio) al *Monas prodigiosa* Ehr.

pag. 106. — Tra le specie fungine che si presume originino il ,,virus'' carbonchioso si fa cenno, sulla fede di NUMANN e MARCHANT, ad un'*Uredo incostans*, ad una *Pucciniola diadelpha* e ad un *Aecidium confertum* non meglio specificati, e indecifrabili.

pag. 270. — Dichiara insussistente la *Tilletia aptogenes* Bender (1869), presunta causa del colera asiatico.

pag. 301. — Circa il *Pilobolus pestis-bovinae* Hallier, presunto agente eziologico della peste bovina, dichiara essere una muffa senza relazione con la malattia suddetta. Pare che sia questa stessa specie quella che a pag. 497 viene indicate come *P. Hallieri* Riv.

pag. 322. — Nega che l'*Oospora rufescens* (Fres.) Sacc. (sub *Torula rufescens* Fres.) si trovi nei foraggi animali, e quindi sia connessa con l'*Ustilago carbo* Tul., come sosteneva HALLIER; non avrebbe quindi rapporti con il vaiolo bovino.

pag. 337—338. — Attraverso delle sue proprie osservazioni, nega che il vaiolo delle pecore si debba alla *Pleospora herbarum*, e che quindi questa specie sia in ,,generazione alternante'' con il *Rhizopus* (,,*Rhyzopus*'') *nigricans* Ehr. e la *Tilletia hollii* Tul. (Di cuest'ultima specie non abbiamo trovato rifereimento nel TULASNE: trattasi forse della *Tilletia lolii* Auersw., publicata nel 1884 (in KLOTZSCH-RABENHORST, Heb. Viv. Mycol., Cent. XIX, N. 1999).

pag. 358—360. — Ricorda e figura, senza commenti, le ,,Alghe'' presunte agenti eziologici della blenorragia (*Crypta gonorroica*) e della sifilide (*C. syphilitica*), con riferimento alle osservazioni del SALISBURY) (1862), nonché all'altro presunto agente della sifilide *Coniotekium syphiliticum* Hallier (*Coniothecium*).

pag. 405. — Pure senza commenti riporta l'opiniore di HALLIER cira i presunti rapporti metagenetici tra *Ascophora elegans* Corda e *Ascotricha chartarum* Berk., che si ammetteva fossero gli agenti di una micosi delle api.

pag. 415. — Si nega ogni valore sistematico al *Cryptococcus clava* WEDL, sulla base di un lavoro dell'HERING.

pag. 415. — Vi si fa riferimento all'*Hormiscium sacchari* Bon. che parrebbe essere un lievito anascosporico, forse blastosporeo od artrosporeo.

pag. 425. — Vi si fa cenno ai giá citati *Tilletia aptogenes* Bender e *T. scarlatinosa* Hallier (vedi pure *Mucor scarlatinosus* Hallier, anche a pag. 496).

pag. 427—428. — Nega ogni valore patogenetico all'*Urocystis intestinalis* Hallier e all'*U. cholerae asiatici* Hallier. Il *Coniothecium stilesianum* Rivolta, dalle belle figure, parrebbe essere un vero *Coniothecium*; sulla fede dello STILES è riportato essere la causa della peste americana dei bovini. Cita nuovamente i *Coniothecium syphiliticum* Hallier e il *C. gonorroicum* Hallier (,,*gonnorroicum*'') (vedi pure a pag. 358—360), mentre nega valore al *Leiosporium dissentericum* Hallier nella genesi della dissenteria dell'uomo. (Il gen. *Leiosporium* sarebbe affine, secondo l'inventore, a *Ustilago* e a *Tilletia*).

pag. 434. — La *Lecythea ruborum* Rivolta é evidentemente la forma uredosporica di qualche *Phragmidium* vivente su un qualche *Rubus*; a pag. 436 ricorderá il *P. incrassatum* DC. (= ? *P. rubi* (Pers.) Wint.).

pag. 435. — Il *Dicoccum tricoilum* Rivolta non é una specie decifrabile, ma non é neppure un *Dicoccum* (cfr. fig. 112b).

pag. 437. — Vi si ricorda una *Puccinia favi* Ardsten, di cui ci é ignota la fonte bibliografica. Piú che la *Puccinia graminiis* accidentalmente trasportata sulle croste di tigne od altre malattie cutanee, ecc.", come in RIVOLTA, potrebbe designare un Dermatofita, di cui sarebbero messi in luce i ,,fusi", una delle forme di closterospore.

pag. 440. — Viene indicato *l'Oidium pulveracearum* Riv. per la *Torula pulveracea* Corda, e l'*Oidium aureum* Riv. per la *Monilia sitophila* (Mont.) Sacc.

pag. 446. — Si designa la *Torula viridis* Blondeau (ubi?) per il ,,*Penicillium glaucum*" (collettiva).

pag. 447. — E'indicato l'*Oidium erysiphoides* con periteci di *Erysiphe* su foglie e fusti verdi di Edera. (Su *Hedera helix* si ha una sola (e dubbia) citazione dell'*Uncinula salicis* Winter; cfr. JACZEWSKI, Mon. Erysiph., pag. 384, 1927). In Italia non si hanno altre segnalazioni di Oidi sull'Edera.

pag. 449. — Parrebbe dubbiamente indicarsi come uno stadio del *Cladosporium herbarum* Corda, lo *Sporodum herbarum* Rivolta, molto mal definito.

pag. 455. — Vengono indicate una serie di specie del genere *Leptomitus* assolutamente indecifrabili, quali *L. Hannoverii* Rob. (che il RIVOLTA identifica con l'*Hormiscium sacchari* Hallier), il *L. epidermidis* Gubler (?), il *L. uteri* Lebert e il *L. oculi* Helmbrecht, per lo piú- secondo il Nostro-miceli sterili.

pag. 481. — La *Mycogone flava* Wallr. (specie non elencata nella ,,Sylloge") vienne indicata sulle spighe di Segala; probabilmente per errore di stampa, il genere viene indicate come ,,*Micogene*" Lk.

pag. 482. — Vi si riferiscono un *Verticillium album* Riv. e un *V. glaucum* Riv. non reidentificabili, nonché un *V. ruberrimum* Riv. detto essere eguale ad uno *Sporotrichum ruberrimum* Rivolta.

pag. 487. — Viene riferito di una *Botrytis reptans* Riv. su culmi di Frumento (fig. 196) indecifrabile, nonché una *B. fusca* Riv. su (soluzioni di ?) citrato di calcio (fig. 197), egualmente indecifrabile.

pag. 491. — Viene dubitativamente indicato un *Cephalosporium macrosporum* Riv. su una Locusta morta chiusa in un vaso, specie detta essere comune sui foraggi. Il disegno della fig. 206 indica abbastanza chiaramente una specie del genete *Monosporium.*

pag. 491—492. — *Periconia phaseoli* Riv. su baccelli di Fagiolo e sul Gelso. Dalla fig. 212 parrebbe una vera *Periconia,* forse non distinta dalla *P. pycnospora* Fres.

pag. 494. — Il *Rhizopus Hallerianus* Riv. (che dovrebbe corrispondere al *Pilobolus pestis bovinae*) viene riferito, dubitativemente, al *Rhizopus nigricans* Ehr.

pag. 496. — Ricorda il *Chaetocladium Jonesii* Fries su feci di Cinghiale e liquido orale di Bue affetto da febbre aftosa.

pag. 498. — Il *Chetostroma betae-vulgaris* Riv., anche dalla fig. 242, parrebbe una *Psilonia* (*Volutella* subg. *Psilonia*). Vegeta sulle radici carnose di Barbabietola, nelle quali indurrebbe un marciume. (A pag. 566) il genere é ricordato, probabilmente per un errore di stampa, come ,,*Chaetastroma*".

pag. 515—517. — Vi si accenna a un *Gutturomyces equi* Riv. che viene detto essere un Aspergillo, ma la fig. 275 induce a credere trattarsi di una Botrytidea.

pag. 520. — Si fa cenno di una *Peziza Phrigerii* del cerume dell'orecchio, con riferimento a GAROVAGLIO (Rnd. Ist. Lomb., Ser. 2, Vol. V, pag. 463, 1872; cfr. Arch. Trienn. Lab. Critt., Pavia, Vol. I, pag. 31, 1874). Notizia su questo curioso ritrovamento fu effettivamente dato dal Garovaglio, che ne promise la descrizione in appendice al Vol. I dell',,Archivio Triennale'', mentre ne pubblicó solamente una bella tavola del Frigerio (ibid., tab. XI). Nella leggenda di detta tavola (ibid., pag. 218) appare il binomio *Peziza auriculae,* che deve essere attribuito al Garovaglio ma come ,,nomen nudum''. Pure senza descrizione il fungo appare come *Peziza Phrigerii* Riv. in Rivolta, l.c., ricordato ancora, pure senza descrizione, in Perroncito (,,I parassiti dell'uomo e degli animali domestici, pag. 81, 1882). La descrizione si avrá soltanto con Cattaneo (arch. Trienn. Lab. Critt. Pavia, Vol. V, pag. 115, 1888) con una nuova iconografia (tab. III, fig. 1—2) e con il binomio *Peziza auriculae* (Rivolta). E' dunque, in realtá, *Peziza auriculae* Cattaneo.

pag. 537. — Ricorda l'*Erysiphe communis* (*Oidium erysiphoides*) sul *Glechoma hederacea.* Potrebbe forse riferirsi all'*Erysiphe labiatarum* Chev. f. *glechomatis* (Dietr.) Jacz.

pag. 541. — Viene indicata la *Pleospora agave* De Not. quale comune sulla paglia esposta alle intemperie, con ascospore 5-settate, e specificamente distinta dalla *P. herbarum* (specie ricordata a pag. 337 in relazione al vaiolo delle pecore secondo Hallier). Potrebbe forse trattarsi della comune *P. vulgaris* Niessl.

pag. 541—542. — *Robergia straminis* Riv. E'forse (fig. 285) l'*Ophiobolus graminis* Sacc.

pag. 544. — Viene ricordata la *Rhizoctonia allii* Grev. quale causa di una malattia dell'Aglio abbastanza bene descritta. Trattasi quasi certamente di uno *Sclerotium.*

pag. 544—545. — Viene indicate la *Peziza cibarioides* Fr. (certamente la *Sclerotinia trifoliorum* Erikss.) sui *Trifolium pratense, T. incarnatum. T. repens e T. hybridum.*

pag. 546—547. — A proposito del *Synchytrium taraxaci* De By. sul *Taraxacum officinale* critica il *S. Mieschierianum* Kuhn, in quanto non ha rapporti con una alterazione delle fibre muscolari del maiale.

pag. 563. — Una *Spicaria solani* Riv. della Patate potrebbe essere (fig. 304) uno *Sporotrichum* o forse pure una *Botrytis.*

pag. 563. — Il *Cylindrotaenium cholerae* Thomé, dalla descrzione e dalla fig. 305, potrebbe essere una specie di lievito filamentoso asporigeno.

On the nature of the so-called "Macroconidia" observed on *Microsporum* infected hairs "in vivo"

by

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(*After a demonstration given at the Meeting of the Chicago Medical Mycological Society, February* 28, 1951)

(3.5.1951)

Introduction

In recent years, in quick sequence, a number of papers have been published by MONTGOMERY et al.,[1] (1948), APPEL and ANSELL,[2] (1949), FELSHER and EIRINBERG,[3] (1950), which brought a most surprising message about the discovery of "true macroconidia" grown in vivo on *Microsporum* infected hairs, i.e. in the parasitic stage both of *Microsporum lanosum* (2) and even of *Microsporum Audouini* (1, 3).

These findings were so extraordinary, and if true, so revolutionary that they would have been able to upset the result of the observations of many during the last hundred years of mycological research and to destroy one of the fundamental experiences, i.e. that dermatophytes in parasitic stage never form organs of fructification, producing them only in their saprophytic life cycle under regular (*M. lanosum*) or only very specific (*M. Audouini*) cultural conditions.

SABOURAUD[4] whose correctness of observation down to the last minutiae in the field of the tineas, is unsurpassed after half a century of the most concentrated and most intensive research, never mentioned having seen organs of fructification in the parasitic stage either of *M. lanosum*, or of *M. Audouini*.

After thirty years of continuous observations made in tineas, the writer found nothing in *Microsporum* infected hairs in vivo (in parasitic stage) which would have incited the faintest suspicion that *Microsporum* fungi in their parasitic stage produce anything like organs of fructification, macroconidia or any other type.

My interest aroused, I decided to check and recheck the validity of the old law, that dermatophytes never produce organs of fructification in their parasitic stage. The possibility that DAVID GRUBY, SABOURAUD (l.c.) and hundreds of outstanding investigators during a whole century could have overlooked such a "detail", appeared extremely slim.

My own investigations were already in progress, and near the closing stage, when AJELLO's[5] excellent paper was published, refuting with good arguments the existence of macroconidia in *Microsporum* infected hairs in vivo, i.e. in the parasitic life cycle of these fungi.

History Of The Present Confusion

Montgomery et al., (l.c.) in 1948, were the first to call attention to peculiar cellular structures in *Microsporum* infected hairs in vivo. In one case of tinea capitis, fuseaux-like bodies on the infected hair shaft were noticed. At different visits of the patient, microscopic examination of a sodium hydroxide preparation of the hair showed these fuseaux-like bodies. Montgomery et al., stated that these structures had not been previously observed at the New York Skin and Cancer Unit. They also referred to verbal communications of Benham, [6] Hopper, [7] and Delamater, [8] who mentioned observing these structures. However, they could find no reference in the literature to the presence of these bodies on direct examination of fungus-infected hairs.

Confronted with the possibility of an exciting discovery, they exercised due caution, declaring that further examination and study is necessary to determine whether these bodies are in reality fuseaux, or whether they may be epithelial cells or other formations which have split off from the cortex of the hair itself.

Their figure 1. represented an infected hair showing a sheath of spores and fuseaux-like bodies at the cortex. They underscored the fact that these bodies are not regularly shaped, nor do they show uniform septa *). In this instance, the culture showed *M. Audouini.*

Second in this series, Appel and Ansell (l.c.) with great enthusiasm, reported on the discovery of "macroconidia" in the parasitic stage of *M. lanosum* (*canis*) in two cases of "tinea capitis". If nothing else, the illustrations of their paper clearly prove that they commited errors in two respects. They did not recognize the true character of the anatomical structure of the hair and hair follicle, and they did not take into consideration a series of well known mycological facts.

In the history of their "discovery" they stated that in 1936, routine examination of a patient with tinea capitis at the Boston City Hospital disclosed on direct examination of an epilated hair objects identified by Dr. John G. Downing [9] as fuseaux. They were also seen by one of the authors (B. A.). The episode caused a "minor flurry of excitement", but unfortunately, the details of the case were lost. Some years later, they stated, the late Dr. Ernst Bernhardt, [10] mycologist of the department of dermatology and syphilology at Boston City Hospital, observed a similar case.

In their first observation, Appel and Ansell found several hair stubs filled not only with a mass of small round spores, but with large, boat-shaped fuseaux, which took the cotton-blue stain deeply. At subsequent visits of the same patient no more "fuseaux" were seen. However, later they observed another "somewhat uncommon phenomenon" in one of the hairs epilated from the patient's sclap. "Long chains of arthrospores, branching dichotomously in the direction of the hair bulb, were present in the shaft of the hair."

In their second observation of "tinea capitis" caused also by *M. lanosum*, they found again in one of the hairs from the scalp, "a single large, typical septate tapered, boat-shaped fuseaux." A search then revealed several hairs filled with fuseaux and with chains of arthrospores.

Appel and Ansell, however, made the following significant statement:

"The morphologic features of many of these fuseaux did not permit of immediate identification. Some did not show clearly the double contour of the wall. Most of them were recognizable only by their outline. The septations and the morphologic character of their cytoplasm were not distinguishable. Whether this state was due to a degenerative change after the fuseaux were once clearly formed or whether these fuseaux were in an aborted phase, is a matter of speculation."

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*) Cf. Fig. 5 which is almost a perfect duplication of their illustration.

APPEL and ANSELL bring two illustrations (figures 1 and 2) from the notes of the late Dr. ERNST BERNHARDT (l.c.). The caption to figure 1 says: "Fuseaux may be found on a typical hair infected with *M. canis* immediately after its epilation. These may have well defined compartments . . . or be somewhat deformed . . ." *)

The caption to their figure 2, also from the notes of the late Dr. ERNST BERNHARDT, reads as follows: "Higher magnification (× 850) of the fuseaux in the center of (their) figure 1. Dr. J. G. DOWNING (l.c.) considered this type of spindle a fuseaux years before a newer staining technic rendered its identity obvious . . . Formely, it was confused with the spindle cells from normal sclaps, seen in preparations containing potassium hydroxide and considered epithelial cells by Sabouraud."

In APPEL and ANSELL's paper their figure 4 is the most amazing. Their caption reads: "Fuseau in fairly sharp focus throughout its entire length, showing thick, double-contoured wall, the tapering, blunt ends and the septations." **) In an inset to this figure they gave an artistic drawing of the same cellular structure described in the caption, which the superficial observer would really be able to confuse with a true macroconidia. Such expressions of the fantasy of an artist, if not so indicated, are not permissible in a scientific publication.

Last in this series is the report of FELSHER and EIRINBERG (l.c.) on the discovery of "macroconidia" on *Microsporum* infected hairs in vivo in "tinea capitis". In their case the culture yielded *M. Audouini*. They found "in addition to numerous ectothrix small spores, many fuseaux, some resembling those of Microsporum and others resembling those of *Trichophyton* . . . While some of the fuseaux appeared ill formed, many were perfectly formed with clearly recognizable septations and definite walls."

Here ends, up to the present, the series of these amazing discoveries comprising a whole chain of unmistakable errors. The most surprising fact remains that they are throughout committed by dermatologists who ex officio should be completely familiar with the microscopical anatomy of the hair. Greater familiarity with mycological structures and facts would have helped to avoid these gross errors.

This confusion should be brought to an end, before a myth may grow old and give ample opportunity to future generations for further discussion of unfounded legends about the existence of organs of fructification (macroconidia or any other kind) in the genus *Microsporum*, or in any other dermatophytes, for that matter, in their parasitic stage. It is believed essential for the clarification of the errors under discussion, to recapitulate, (1) the anatomical facts about the finer structure of the hair and hair follicle and (2) the fundamental mycological facts.

I. *Anatomical facts about the finer structure of the hair and hair follicle* (6, 7).

The hair follicle itself is a tiny, connective tissue "bag" lined with the layers of the epidermis as it dips down into this cavity, with the exception that it loses one layer, the stratum corneum, and basically is represented by the rete mucosum alone.

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*) Cf. Similar structures can be seen in the figures 6—10 of this paper.

**) Cf. In the figures 5, 6, 13, dozens of similar cells are present.

The hair itself has two basic portions: (1) the "aerial" portion, protruding from the hair follicle, above skin level, — the hair shaft (scapus pili); (2) the "hidden" portion, firmly embedded in the hair follicle, extending from the opening of the hair follicle down to the hair papilla, — the hair root (radix pili). The hair root is sitting with its concave end, the hair bulb, upon a mushroom-shaped structure, the hair papilla.

The root of the hair consists of six concentric layers. Three of them constitute the hair proper: (1) medulla, (2) cortex, (3) cuticle (cuticula pili); the external three layers form the internal root sheath consisting of (1) cuticle (cuticula vaginae), (2) Huxley's layer and (3) Henle's layer.

Within the hair follicle the hair (strictly the hair root) consists of all six of these layers. However, at the upper end of the follicle, just below the opening of the duct of the sebaceous gland, the three layers of the internal root sheath become loose and disintegrated, and thereafter, the solid hair emerges from the hair follicle consisting only of its own three layers.

The cuticle of the hair (cuticula pili) covers the hair shaft as a thin, semitransparent, shiny membrane composed of flat, imbricated cells, not unlike the shingles of a roof, with the difference being that the open end of these imbricated cells point toward the end of the hair. This structural arrangement plays an important role in the invasion of the hair shaft by the fungi. The cortex of the hair shows a fibrillary structure easily recognizable when the invading mycelia of the fungus break up the hair shaft.

The confusion about the existence of "macroconidia" in the parasitic life cycle was prompted by the faint resemblance of the cells of Henle's layer to the true macroconidia. The latter have, indeed, the form of a spindle (fuseau) cut apart in the vertical plane.

But how is it possible, as MONTGOMERY et al, (l.c.) have stated, that these "macroconidia" never were seen before in their Clinic? The important surprising factor lies in the way of epilation of the fungus infected hair.

If the hair is still living (growing), i.e. the hair is a "bulb hair" (German: Papillenhaar, French: cheveux à bulbe creux), it is firmly anchored in the hair follicle, although the hair shaft and the hair root may be completely invaded by the fungus. Because of this the slightest pull on the hair shaft causes it to be broken off somewhere *above* the follicle opening. Thus, the observer will be confronted only with the three layers of the hair shaft: cuticle, cortex, medulla (if present).

If the disintegration of the *Microsporum* infected hair shaft has progressed far, then one sees on it fragments of the spore sheath, the disintegrating cells of the hair cuticle as they fall out and apart from their structural connection, and the hair shaft split off in its fibrils.

However, if one happens to epilate a "dead hair," no longer growing but still captive in the follicle, the observer is confronted with an entirely different situation. This "dead hair" (German: Kolbenhaar, French: cheveux à bulbe plein), a "club hair" will suddenly present, structures unfamiliar and long forgotten, comprising the element of surprise and of the unusual. Such a "club hair" presents in its root portion all six layers, in other words, the hair root with the inner root sheath. Its outermost layer is Henle's layer.

Since the cell structure of Henle's layer had already undergone a complete disintegration within the follicle, the traction at the epilation, and later the mounting fluid (chlorallactophenol) complete the final disintegration of this cell layer. Thus, the cells of Henle's layer are scattered irregularly over the whole root or they lie together covering small areas beside or in the vicinity of the hair.

For the exact observer the most conspicuous fact must be that these spurious "macroconidia" are lying and can be found only at the root portion of the hair. Distal from the follicle opening these "spindle-shaped" cells, of course, never can be found. (Cf. hair in figures 4 and 5).

The shape of these fuseau-like structures is fairly uniform; they are tapered off at the free edge (distal), while the proximal end still shows the rest of the attachment to the proximal cell. Mostly they seem to be "unicellular". A few, however, show some transverse lines which at first sight can be mistaken as "transverse walls" or "septa". Some show only one, others 2 or 3 transverse lines giving the false impression of a multilocular cell, a macroconidia, (Cf. fig. 9). It becomes clear at further observation, however, that these spurious septa never go from cell wall to cell wall as do true septa. They are completely irregular as to their length, position and marking. At random, fifty of these cell structures have been measured. The minimum size measured 26.6 μ ⩔ 11.4 μ; the maximum 38.0 μ ⩔ 11.4 μ; the average was 32.7 μ ⩔ 10.2 μ. These measurements closely match those of AJELLO (l.c.).

These are the anatomical facts which dermatologists should try to remember before they confuse animal structures with those of vegetable origin.

Thus, it must be completely clear that the observer will have in his preparation the spindle-shaped cells of Henle's layer, no matter whether the hair is fungus-infected by fungi of any kind, or a normal, healthy one, *as long as the hair root is epilated along with the internal root sheath.*

II. *The Fundamental Mycological Facts.*

In order to avoid confounding the spindle-shaped cells of Henle's layer with true macroconidia of the genus *Microsporum*, the following criteria are offered:

(1) *Refractivity.* — Looking at a "club hair", thoroughly infected with *Microsporum* (whether zoophilic or anthropophilic does not matter) in a wet mount prepared with chlorallactophenol, the first important difference between animal and vegetable structures is the difference in their refractivity. The vegetable (fungus) structures reveal a much higher refractivity and a conspicuous greenish hue compared with the flat, dead-gray color of the animal cells. The cells of Henle's layer reveal the latter characteristics.

(2) *Staining properties.* — The spurious "macroconidia", being animal structures, took up anilin dyes like cotton-blue avidly and with uniform density. (Cf. figs. 8, 9, 10). Small spores around the hair or true macroconidia from the culture of *M. lanosum* did not take the cotton-blue uniformly and completely; if they took the stain, then only in specific parts of the macroconidia. Unstained true macroconidia reveal a thick, homogenous (double-contoured) wall with highly refractive transverse septa. The multilocular cells (8 to 12 in the average) are not homogenous; their content is granular. In stained true macroconidia (cotton-blue) the thick wall and the transverse septa take the anilin dye only very faintly or not at all, whereas the multilocular cells (their contents) are uniformly deeply stained. The contrast is very conspicuous. The difference is between the animal (dead) cells — altogether completely cornified elements — and the vegetable (fungus) structures (living elements) which take up the dye selectively or not at all.

(3) *Cultural characteristics.* — It is common knowledge that spores of any kind (macro- or microconidia) swell before germinating. The spurious "macroconidia" never revealed any swelling in the culture chamber (van

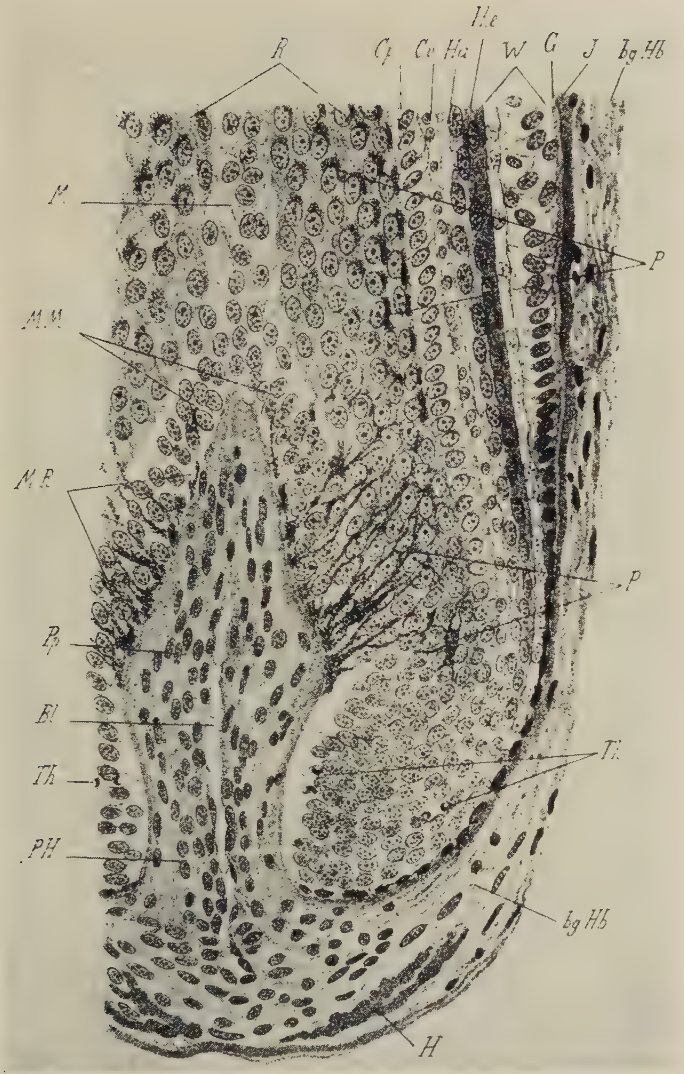

Fig. 1 *) (31). Longitudinal section through a papilla, bulb and lower portion of a hair and its sheaths from the scalp. Pp. papilla, P. H. neck of the papilla, Bl. blood vessel, M. cells of the medulla of the hair, R. cortical cells of the hair, M. M. matrix cells of the medulla, M. R. matrix cells of the cortex, Th. mitotic figure, P. pigment, C. p. cuticle of the hair, C. v. cuticle of the internal root sheath, Hu. Huxley's layer, He. Henle's layer, W. the external root sheath, G. the glassy layer of the hair bulb, T. the innermost layer of the connective tissue of the hair follicle, bg. Hb. connective tissue of the hair follicle, H. Kollastin. Magnification 300 ×.

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*) Figures 1 (31), 2 (32), 3 (33—34) are reproductions from Mraček's Handbuch (l.c.) on pages 62, 67, 68, respectively.

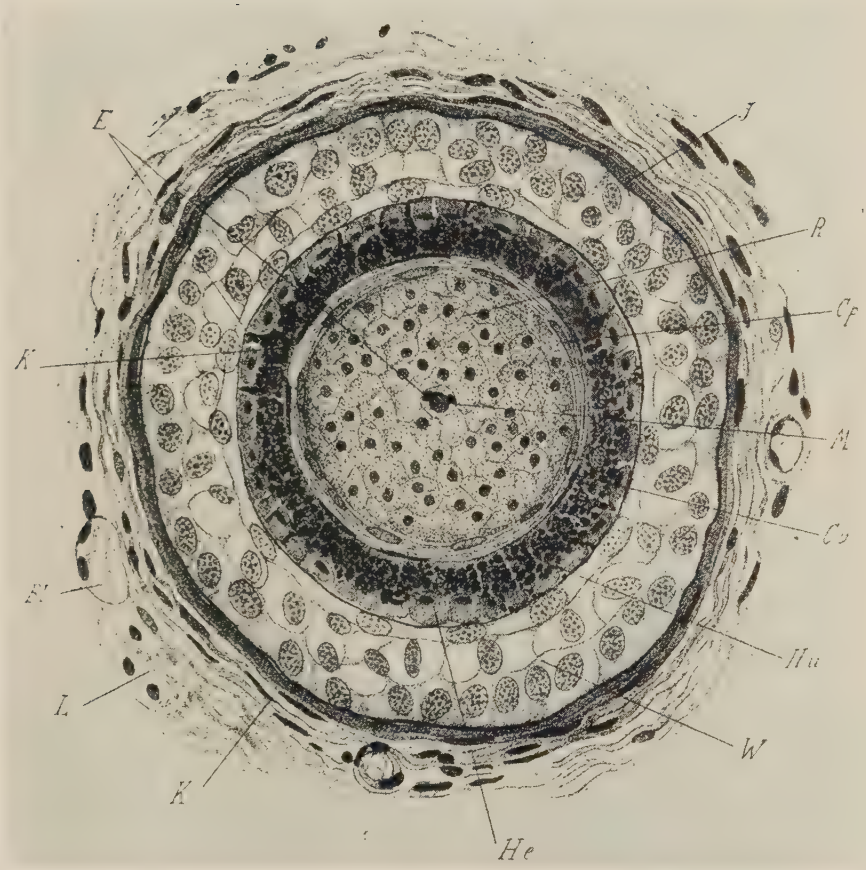

Fig. 2 (32). Cross section through a hair with its sheaths and the hair follicle from the scalp. M. cells of the medulla, E. Eleidin granules, R. cells of the cortex of the hair. The five dots are cross section of the hair fibrils. C. p. cuticle of the hair, C. v. cuticle of the internal root sheath, Hu. Huxley's layer, K. (in the center) nucleus of one of the cells, He. Henle's layer, W. the external root sheath, T. glassy layer, K. (left below) layer of circular fibrils, L. layer of longitudinal fibrils, Bl. blood vessel. Magnification 500 ×.

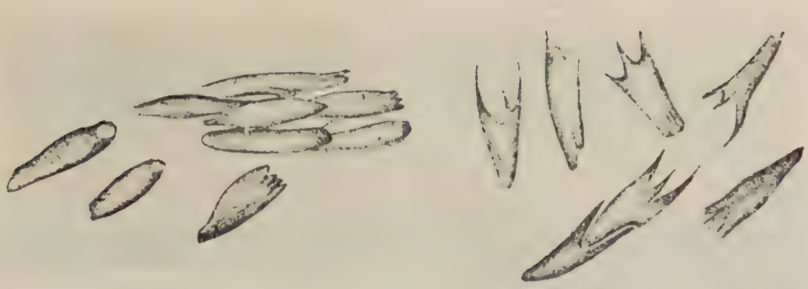

Fig. 3 (33, 34). Left (33) isolated cells of Henle's layer of the internal root sheath of a hair of the beard. Right (34) isolated cells of Huxley's layer of the internal root sheath of a hair of the beard.

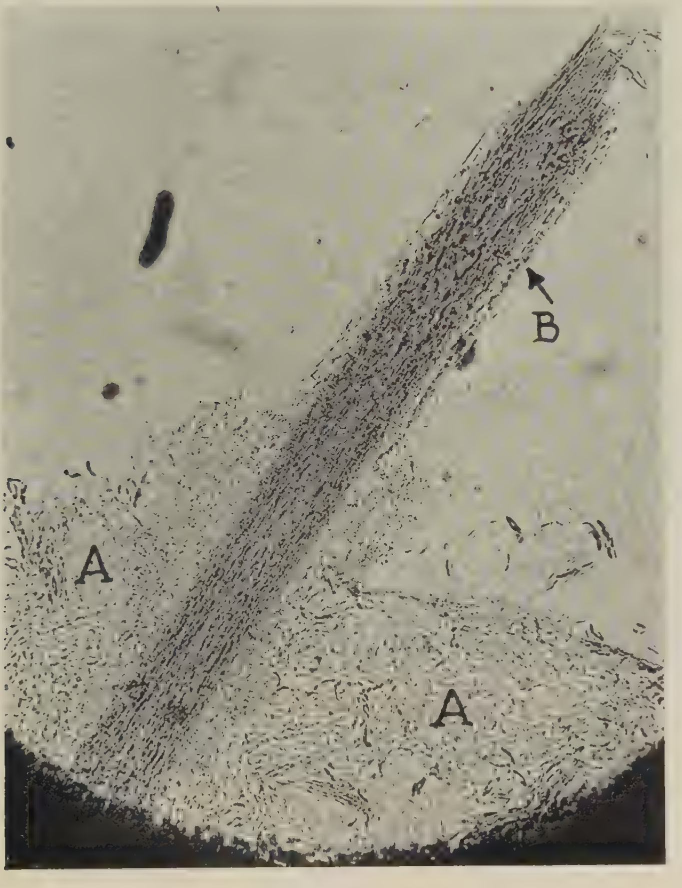

Fig. 4. *Microsporum* infected hair (*M. lanosum*). A. cells of the follicle epithel intermingled with fragments of the spore sheath. B. row of disintegrating cells of the hair cuticle (cuticula pili). Wet mount in chlorallactophenol, × 300.

*) Where not stated otherwise, the illustrations are original photomycrographs of the author.

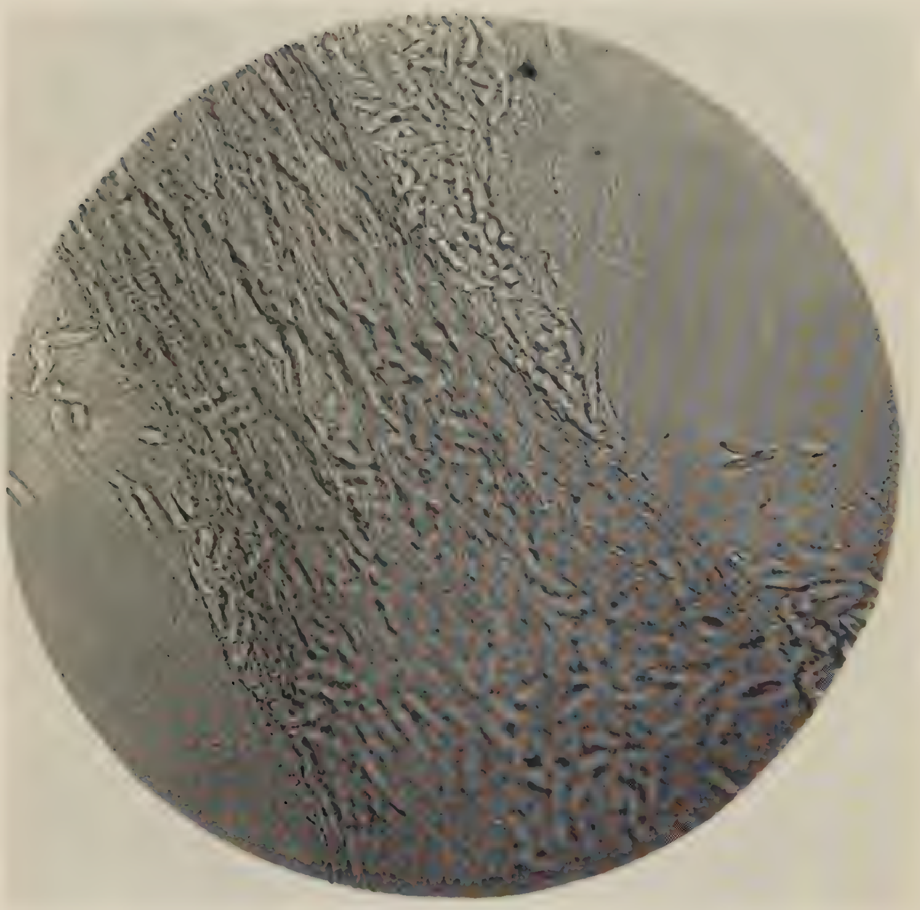

Fig. 5. Bulbar portion of a *Microsporum* infected hair (*M. lanosum*), showing disrupted rows of cells of Henle's layer. At the edges these cells are in focus, upon the hair they are out of focus, but they give a good idea, how these cells cover the hair root all around. Wet mount in chloral-lactophenol, × 500.

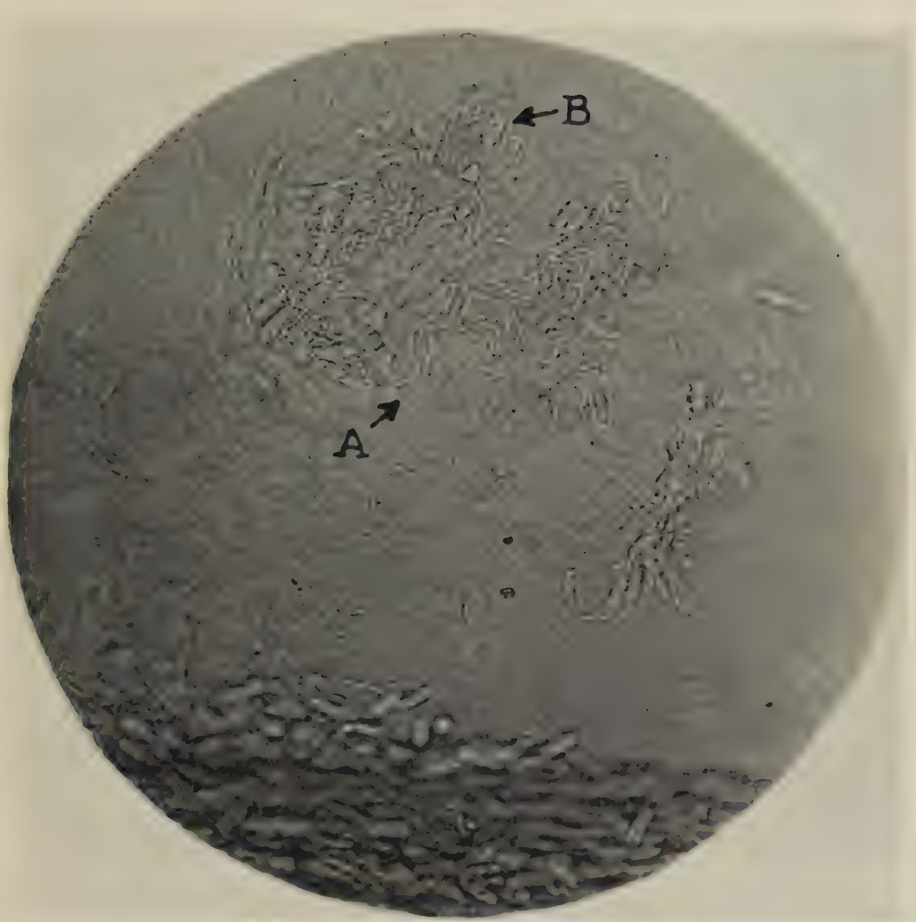

Fig. 6. Follicular portion (upper third) of a *Microsporum* infected hair (*M. Audouini*). Below is the portion of the hair, covered with disrupted rows of cells of Henle's layer. Above there is a patch of disrupted cells of Henle's layer intermingled with fragments of the spore sheath. Many of these cells are imitating "macroconidia", some of them showing even spurious "transverse septa" at A. and B. Wet mount in chlorallactophenol, × 500.

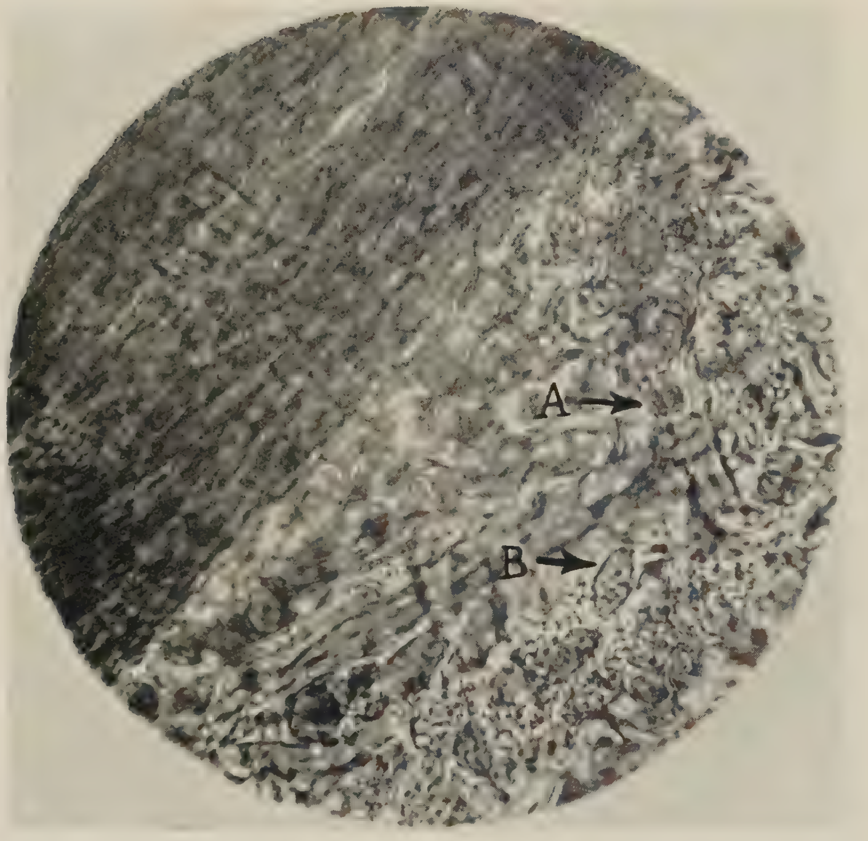

Fig. 7. Follicular portion (upper third) of a *Microsporum* infected hair (*M. Audouini*). Left is the hair. Right: discrupted rows of cells of Henle's layer intermingled with fragments of the spore sheath. A and B isolated cells of Henle's layer imitating "macroconidia". In A one spurious "transverse septum" is clearly visible. Wet mount in chlorallactophenol-cotton-blue, × 1111.

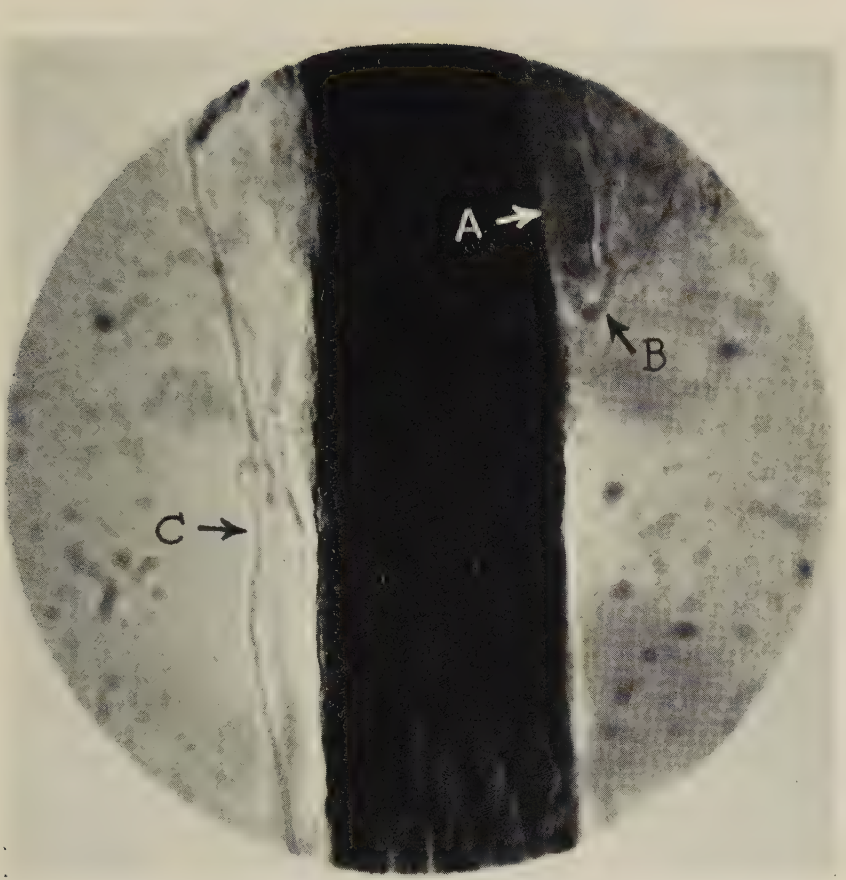

Fig. 8. Follicular portion (upper third) of a *Microsporum* infected hair (*M. lanosum*). A. one isolated cell of Henle's layer with two spurious "transverse septa", deeply stained, imitating a "macroconidia". B. invading piece of mycelium winding around the cell A, growing downwards into the follicle. C. invading piece of long mycelia (one in focus, another behind out of focus) with dichotomous branching, growing downwards into the follicle. Wet mount in chlorallactophenol-cotton-bleu, × 1111.

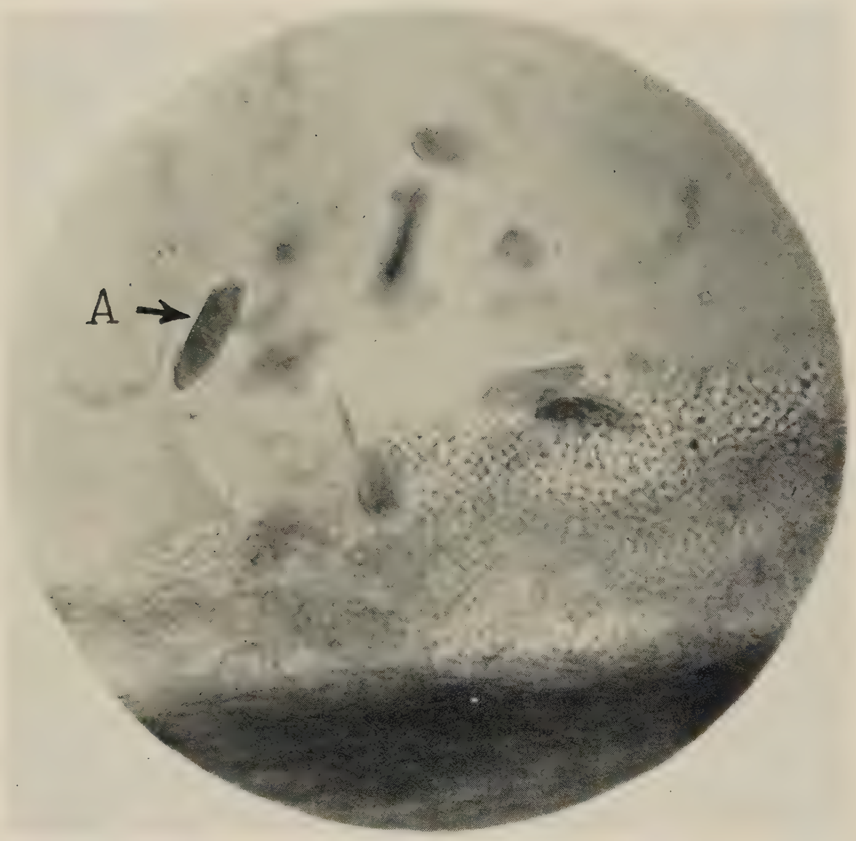

Fig. 9. Follicular portion (upper third) of a *Microsporum* infected hair (*M. Audouini*). Below is the hair upon which a thick layer of the spore sheath is visible. Above the latter there are several typical "tailed" cells of Henle's layer. The cell in A is imitating a "macroconidia" with several spurious "transverse septa". Wet mount in chlorallactophenol-cotton-blue, × 1000.

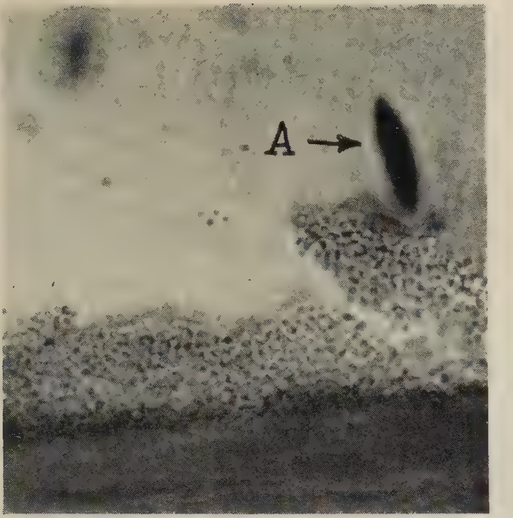

Fig. 10. Follicular portion (upper third) of a *Microsporum* infected hair (*M. lanosum*). Below is the hair, upon which a thick layer of the spore sheath is visible. In A there is an isolated cell of Henle's layer, perfectly spindle-shaped, imitating a "macroconidia". (Compare the differences in the staining between hair and cell of Henle's layer, animal structures on one hand, and the small spores of Microsporum, vegetable structures, on the other hand). Wet mount in chloral-lactophenol-cotton-blue, × 1000.

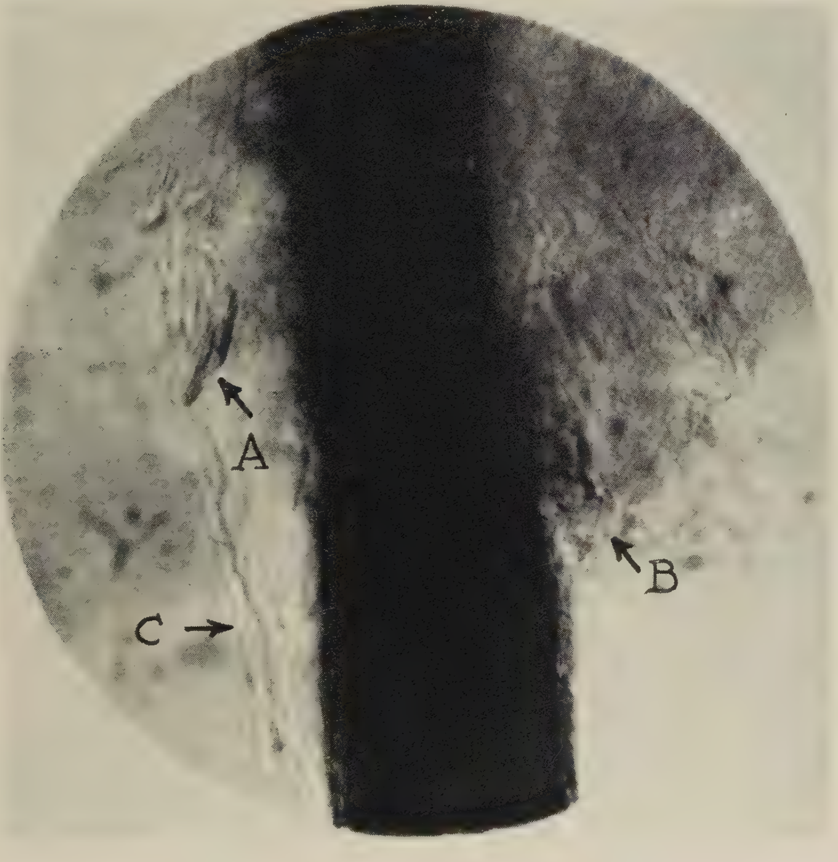

Fig. 11. Follicular portion (upper third) of a *Microsporum* infected hair (*M. lanosum*). At A there are two connected cells of the disrupted Henle's layer (Cf. fig. 3 (33), imitating "macroconidia". In B and C gross mycelia are growing downwards into the follicle. Wet mount in chlorallactophenol-cotton-blue, × 1111.

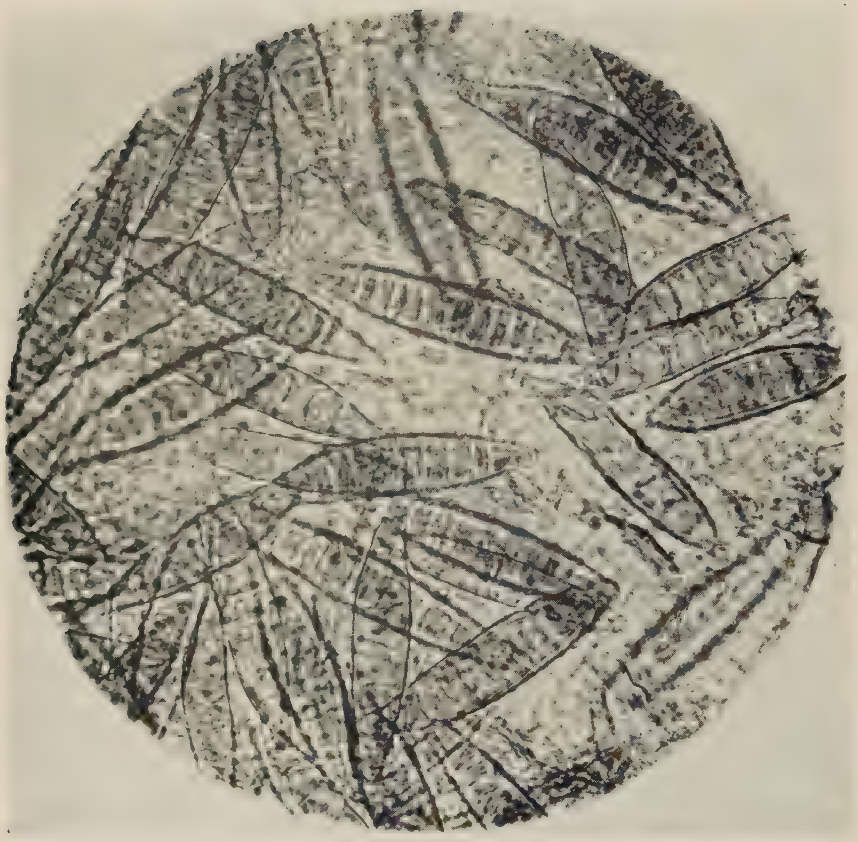

Fig. 12. From a culture of *M. lanosum*, 2 weeks old on Sabouraud's glucose agar. (Cf. with fig. 13). These true macroconidia have a "body", they are sharply delineated, with regular, symmetrically spaced transverse septa, running from wall to wall. Their size is a multiple of that of the cells of Henle's layer. Wet mount in chlorallactophenol-cotton-blue, × 1111.

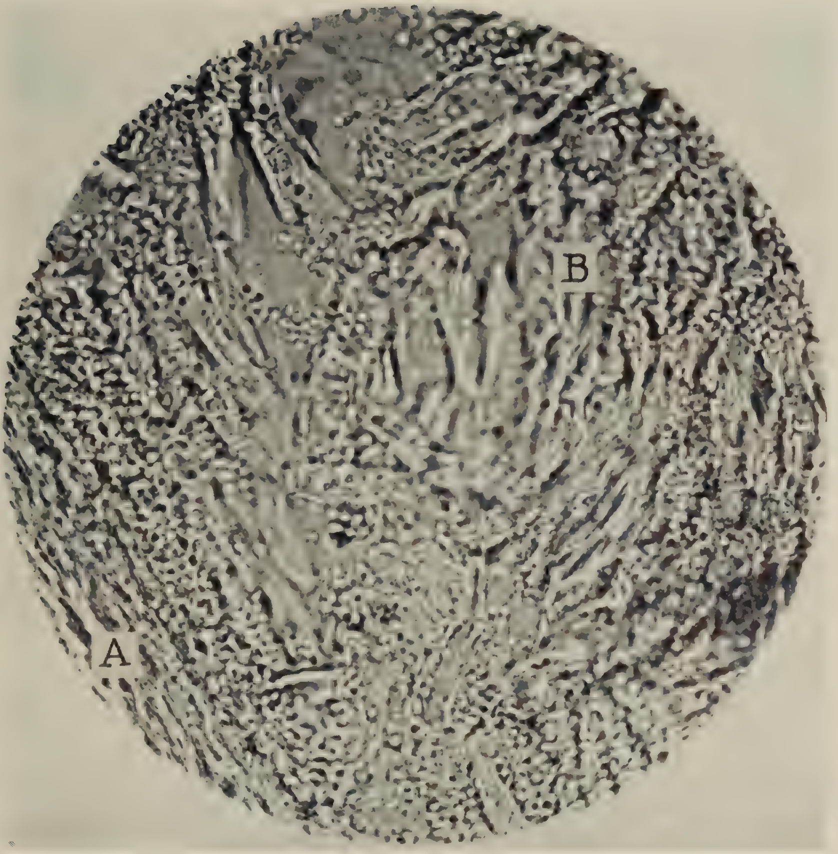

Fig. 13. Follicular portion (upper third) of *a Microsporum* infected hair (*M. lanosum*). A is the portion of the hair. B disrupted cells of Henle's layer intermingled with fragments of the spore sheath. Some of these cells at B are imitating "macroconidia". Wet mount in chlorallactophenol-cotton-blue, × 1111.

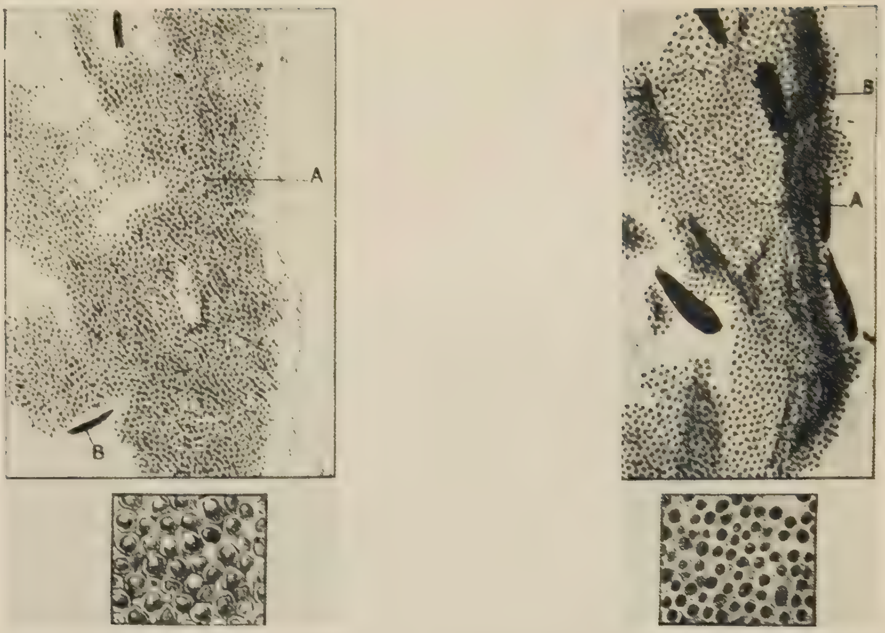

Fig. 14 (13, 14) *). Left (13): Fragment of the spore sheath of a *Microsporum* hair. In A is the spot represented in the lower square, × 750; B is an epidermal cell. Without coloration, × 260. Right (14): Fragment of the spore sheath of *M. audouini*. A is the spot represented in the lower square, × 750. B is an epidermal cell. (Blue of Sahli, × 260).

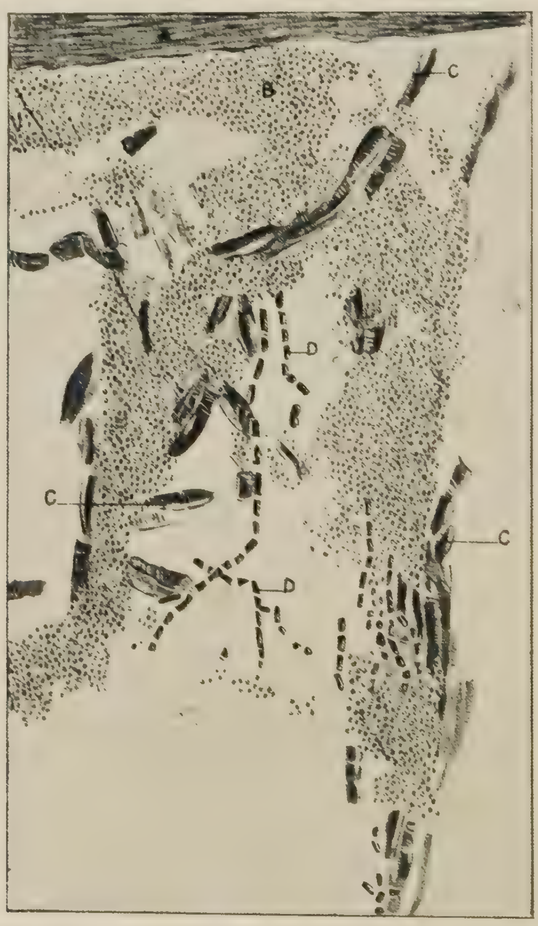

Fig. 15 (69) *). Fresh mount in a case of tinea microsporina caused by *M. lanosum*. A is the hair, B is the spore sheath from which fragments drop down intermingled with epidermal cells (C.), and with the invading gross mycelium (D.), × 260.

*) Figures 14 (13, 14) and 15 (69), are reproduced from Sabouraud's Les Teignes (l.c.), on pages 161 and 222, respectively.

Tieghem cell). In contradistinction, the microconidia (cells of the spore sheath around the hair) swelled and germinated.

Macroconidia of *M. lanosum* in a van Tieghem cell, mounted in glucose broth, swelled and germinated easily in 16 to 18 hours. Needless to say, the cells of Henle's layer never did so in any length of time.

(4) *Comparative measurements.* — The resemblance between the true macroconidia and the spindle-shaped cells of Henle's layer is extremely faints both in shape and in size. The cultural size of the true macroconidia of *M. lanosum* measured, in growth in a van Tieghem cell, a minimum of 57 µ ⩔ 11.4 µ and the maximum of 76 µ ⩔ 15.2 µ. Some dwarf forms of macroconidia measured 38 µ ⩔ 15.2 µ. These figures are also in accordance with Ajello's (l.c.) measurements. In comparison, the average size of the spindle-shaped cells of Henle's layer measured 32.7 µ ⩔ 10.2 µ, even considerably less than the dwarf forms of true macroconidia.

The spindle-like cells of Henle's layer compared with true macroconidia have no "body". They are thin, flat, two-dimensional, while true macroconidia show at first glance a "body", definitely tri-dimensional.

(5) The really amazing feature in this confusion is that those who discovered the "macroconidia" in the parasitic life cycle of these fungi did not even look for sporophores on which these organs should arise. One basic requirement for any organs of fructification is that they do not grow in "mid-air." They must have some connection with their thallus: they do grow on specialized structures, the sporphores. However one may crush a little piece of a culture of *M. lanosum* (or of any other fungus with differentiated organs of fructification) they will still show plenty of their organs of fructification connected with their sporophores or showing, at least, the portion of their attachment at their proximal end.

The spindle-shaped cells of Henle's layer have no connection with any kind of mycelial elements like sporophores and they reveal no trace of a similar attachment.

Comments

Although it is not closely connected with our main problem, the false assumption of Apel and Ansell (l.c.), and of Felsher and Eirinberg (l.c.) of the existence of true macroconidia in the parasitic stage of the genus *Microsporum*, traceable to meager anatomical and mycological information, it becomes necessary to comment on another statement of Appel and Ansell to prevent the rediscovery of other firmly established knowledge, already over one hundred years old.

Appel and Ansell underscored as an "uncommon phenomenon", "the long chains of arthrospores branching dichotomously in the direction of the hair bulb." For those thoroughly familiar with the mode of infection of the scalp by fungi, their statement contains nothing new or extraordinary. It has been stated that new is only what was forgotten. David Gruby in 1841—44 and Sabouraud (l.c.) in 1910, described most remarkably the mode of infection of the hair in "tinea capitis". Suffice it to state again that the hair as such is never primarily infected, rather the fungi first take hold in the "stratum corneum" of the epidermis of the scalp. When in their parasitic vegetation they reach a hair follicle they firmly establish a whole colony of heavy mycelia in and at the ostium of the follicle. From here the mycelia dip down into the funnel of the follicle and then in contact with the hair invade the cortex, entering at the open end of the cuticle cells ("cuticula pili"). These mycelia grow downward, dichotomously branching (Cf. figs. 8 and 11) and splitting up in large arthrospores. In the lower third of the hair root one may find then only the typical well known small spores in mosaic pattern, which does not allow the reconstruction of the mycelia from which they derived.

Whether one finds heavy mycelial threads or arthrospores around the epilated hair depends entirely on the technic of epilation and the duration of the existing infection. If the follicular portion of the hair can be examined, one can easily and often find invading mycelia near and around the follicular ostium and just below it. If they are "young" they are continuous mycelial threads with regular transverse septa, (Cf. Figs. 8 and 11). If they are older, they break up into arthrospores.

Already AJELLO (l.c.) has refuted the statement of APPEL and ANSELL (l.c.) quoting BERNHARDT (l.c.) and DOWNING (l.c.), who claimed that SABOURAUD (l.c.) confused (!) "macroconidia" with cells of Henle's layer. These authors claimed that SABOURAUD had already seen the macroconidia in the parasitic life of the genus *Microsporum*, but he (SABOURAUD) confused (!!) it with "epithelial cells".

Figures 14 and 15 clarify the error of APPEL and ANSELL, BERNHARDT and DOWNING. The "epithelial cells" depicted are disintegrated cells of Henle's layer which SABOURAUD did not specify as such. In SABOURAUD's illustration (fig. 15) we see the most accurate and graphic presentation of the spurious "macroconidia" of APPEL and ANSELL, with even the "transverse septa" present.

FELSHER and EIRINBERG (l.c.) went so far as to state that some of their spurious "macroconidia" showed a resemblance at times to spindle-spores of *Microsporum*, sometimes to those of *Trichophyton*. This statement contains double misinformation. So far as the true fuseaux of the genus *Microsporum* and the genus *Trichophyton* are concerned, they are so different, their similarity so slight, that these organs of fructification form the basis of their generic separation and classification.

My investigations completely confirm the results of AJELLO (l.c.). He correctly recognized that the so-called "macroconidia" of the authors under discussion are not vegetable cells but are of animal origin. A slight correction which must be made, is that the cells of animal origin are not cells of the "inner root sheath "cuticle" but cells of Henle's layer. The cuticle of the inner root sheath ("cuticula vaginae") consist of low (from upside downward), broad (around the follicle) and thin (from outside inward), bright cells. They are never spindle-shaped. In AJELLO's paper the figures 1 and 2 correctly show the cells of Henle's layer.

Summary

1. The so-called "macroconidia" observed on *Microsporum*-infected hairs "in vivo" is an animal cell and not of vegetable origin.

2. The cells described as "macroconidia" in the parasitic stage of the genus *Microsporum* are typical cells of Henle's layer of the internal root sheath of the hair.

3. Whether these spurious "macroconidia" can be seen in a preparation or not depends entirely on the mode of epilation.

4. The cells of Henle's layer can be easily differentiated from true macroconidia by a number of criteria, such as refractivity, staining properties, cultural characteristics, comparative measurements and last but not least, they have no connections with any mycelial elements.

5. SABOURAUD correctly recognized these spurious "macroconidia" as epithelial cells.

6. One of the basic laws of mycological observation that dermatophytes do not produce any organs of fructification in their parasitic life cycle, remains unchanged.

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Notes on the Trichophytoneae from Java

by

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(10.6.1951)

Introduction

The *Trichophytoneae* comprises a group of the *Deuteromycetes*, which is chiefly growing on skin, hair, nails and feathers. Up to now no perfect fructifications are known, though it is very probable, that they may belong to the *Gymnoascaceae*. Especially in the genus *Ctenomyces* we find the same type of aleuriospores and spiral-hyphae as are known for a number of the *Trichophytoneae*. But to place certain of our forms in the genus *Ctenomyces*, as is done by Langeron (14) in my opinion is not allowed. If at some time a perfect stage will be encountered, it most probably will show to be a new though related genus.

Furthermore it is usage to designate the conidial fructifications with a separate name. The name *Ctenomyces* therefore cannot be applied to members of our group.

As was already stated our fungi belong to the *Deuteromycetes* and consequently only asexual spores are formed. In some species up till three different kinds of spores may be observed.

First there are the aleuriospores, one-celled conidia, which are laterally formed on the hyphae.

They are mostly very abundant, in many species giving rise to a powdery appearance of the cultures. The second spore-form is the so called closterospore, a large septated spore arising either laterally, terminaly or even intercalary on the mycelial threads. The last spore type is the chlamydospore, which can be found in all known species. It is a kind of resting spore, rather large, globose till elongated, with a more or less thickened cell-wall and a granular contents. They may arise terminally, laterally or intercalary on the hyphae. Sometimes rows of hyphal-cells are transformed into spore-like bodies, in which case the term arthrospores is often used. But they are no true arthrospores but actually only chains of young chlamydospores.

Whereas in some species all three spore-forms are to be met with, in others only two of them are formed and there are a number of species, which show only one spore-form, viz. the chlamydospore.

However those species are nearly related to the others with more spore-forms. When namely large quantities of cultures are made, as was done by Langeron (14) for *Achorion Schoenleini*, in some of the cultures aleuriospores could be found. In *Achorion concentricum* I found a small number of aleuriospores in one of the primary cultures. But they readely dis-appear in the subcultures.

We have to deal therefore with a gradual reduction of the number of spore-forms. The mycelium in all species is mostly rather uniform, but some are showing spiral-hyphae. In some species hyphae with swellings near the crosswalls (Mycelium en raquette) are often found. Also hyphae with small exerescences (pectinate hyphae) are met with in a number of species. Both types of hyphae however seems to have no taxinomical value. In a few species at last so-called nodular organs are found, which in my opinion are only degenerated ascogonia.

In the course of time 26 genera have been proposed to accomodate the different species of the *Trichophytoneae*. Many of these names have been published with very scanty data or without a botanical description at all. So in many instances it is often very difficult or even nearly impossible to find out what was really meant with some of those names.

LANGERON (14) has reduced the number of genera to four, viz. *Ctenomyces*, *Sabouraudites*, *Epidermophyton* and *Trichophyton*.

As I have mentioned already, the name *Ctenomyces* cannot be applied. The same is true for *Sabouraudites*, as the older name *Microsporum* has priority.

By making use of cultural and especially morphological characters I now propose to divide the *Trichophytoneae* into 6 genera; which are all to be found in our region.

In the literature about 150 species and varieties of our fungi are enumerated.

What was said concerning the genera is also true for the species. A large part of the descriptions has no value whatever, as the essential data are wholly lacking.

Furthermore a large number of names are only duplicates, as owing to the poor diagnoses, species were repeatedly provided with a new one. At present it is difficult to estimate the actual number of species, but according to LANGERON (14) there are about 20. Judging from the experience made in Java, only a small number of forms is constantly met with, mostly the same species as found in other parts of the world.

In the classification of all fungi the microscopical characters are of paramount importance and it is on those lines, that the study of the *Trichophytoneae* has to be tackled. Clinical data so frequently used in the study of our group, cannot serve our purpose.

The study of the giant-colonies may furnish also some data, but they are mostly of secondary importance. We may distinguish between cottony, velvety, feltlike, powdery or even glabrous colonies. The latter type of colony according to LANGERON (14) is an artificial product, caused by the culture media. That may be true, but all colonies of the *Trichophytoneae* on the usual media are more or less artificial. The media on which our furgi are mostly cultivated are those of SABOURAUD or some alteration.

It is quite possible, that in the future new media will be devised on which a more natural growth can be produced. The application of specific vitamins seems promising, also for the development of the complete morphology. But the fact, that on the same media different colonies are formed by certain species is as it were a demonstration of specific or even generic differences, even if the mode of growth is only a modification of the normal type.

Of all the species mentioned in this paper pure cultures were made, and a number of those have been deposited in the collection of the "Centraalbureau voor Schimmelcultures, Baarn, Netherlands." The remaining ones however were lost during the war.

All the material was collected at the department for Dermatology of the University of Indonesia, Djakarta (Batavia).

The color notes were made with the aid of R. RIDGWAY's Color standards and color nomenclature, Washington 1912.

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The genera and species.

A genus, which takes a separate place and is sharply defined from all others, is the genus *Microsporum*. It is characterised by its fusiform closterospores, provided with a thick asperulate cellwall.

When in the remaining genera closterospores are present, they are typical cylindric, smtooth and thinwalled.

In this series of more or less allied forms, the genera are distinguished according to the number of spore types present.

KEY TO THE GENERA

1 Closterospores present.
  2 Closterospores thickwalled, asperulate *Microsporum*
  2 Closterospores thinwalled, smooth
    3 Aleuriospores absent *Epidermophyton*
    3 Aleuriospores present
    4 Closterospores small, spiralphyphae present *Ectotrichophyton*
    4 Closterospores large, no spiralhyphae *Sabouraudiella*

1 Closterospores absent.
    5 Aleuriospores and chlamydospores *Trichophyton*
    5 Only chlamydospores *Achorion.*

When all comes to all it is a matter of taste if one will consider our groups, with the exception of *Microsporum*, as genera or only as the representatives of one rather variable genus. In this instance we have to select the oldest name for this large genus viz. *Trichophyton*. All other names can then be used for the division of the genus into subgenera. But for the time being it seems advisable to consider them as formgenera.

Microsporum GRUBY C. R. Acad. Sci. Paris 17. 301—303. 1843.
Lophophyton MATRUCHOT et DASSONVILLE Rev. Gén. Bot. 11. 429—444.1899.
Pinoyella CASTELLANI et CHALMERS Man. Trop. Med. ed. 3. 1023. 1919.
Sabouraudites OTA et LANGERON Ann. Parasitol. Hum. Comp. 1, 326—329. 1923.
Closterosporia GRIGORAKIS Ann. Sc. Nat. Bot. 10. 7. 411. 1925.

Colonies mostly large and velvety, with small elevated centre. Growth rapid. Mycelium producing some spiral hyphae, especially on suitable media. Aleuriospores present. Closterospores mostly abundant, fusiform many septated, thickwalled and provided with asperities, sometimes only at the poles. Chlamydospores always present. Type species MICROSPORUM AUDOUINI GRUBY.

MICROSPORUM FELINEUM FOX et BLAXALL Brit. Journ. Derm. Syphilis. 10. 354. 1896.
Microsporum canis BODIN in GUÉGUEN, Champ. Paras. Homme Anim. 144. 1904.
Microsporum lanosum SABOURAUD Ann. Derm. Syphilogr. IV. 8. 172—183, 225—245, 1907.

Microsporum caninum SABOURAUD Ann. Derm. Syphilogr. IV. 9. 153—156. 1908.
Sabouraudites lanosus OTA et LANGERON Ann. Parasitol. Hum. Comp. 1, 329. 1923.
Closterosporia felinera GRIGORAKIS Ann. Sc. Nat. Bot. X. 7. 415. 1925.
Closterosporia lanosa GRIGORAKIS Ann. Sc. Nat. Bot. X. 7. 415. 1925.
Microsporum niveum CARUSO Giorn. Ital. Derm. Sifilol. 67. 278—280, 1926.
Sabouraudites felineus LANGERON et MILOCHEWITCH Ann. Parasitol. Hum. Comp. 8. 491—492. 1930.

Rapid growing flat colonies with small central elevation, sometimes with a few indistinct radial furrows: velvety, central parts sometimes granular, margin radiating. Colour at first white, afterwards pale yellowish, about cartridge buff. On the reverse colour af first yellow, about between Antimony yellow and yellow ocher, afterwards brown, between lawny and russet.

Mycelium richly branched and septated, the separate threads 1½—6 μ broad. On the thin hyphae the aleuriospores are originating, especially in young cultures. They are laterally situated, sessile, hyaline, slightly clavate with truncated base, 3½—6 × 2 — 2½ μ. From the thick hyphae many side branches are originating under nearly right angles.

Some hyphae have undulating walls, others are showing swellings near the crosswalls.

Spiralhyphae always present, though often scanty. On the larger hyphae the closterospores are formed, either terminally or intercalary, sometimes one after another. They are very variable as to shape and dimensions, colourless till pale yellowish, fusiform till elliptic-fusiform, poles often drawn out, mostly straight, seldom unequal-sided, thickwalled, the wall thinning out near the poles, covered with colourless asperities, often only at the poles, seldom wholly smooth; with 1—11 mostly 5—7 crosswalls, 35—83 × 8—21 μ. Most common dimensions 58—70 × 14—17 μ. Wall 1—3 μ mostly 2—2½ μ broad.

Chlamydospores not abundant, globose till elongated, with slightly thickened cellwall, terminal or intercalary, 10—14 μ in diam. or 11½—16 × 9½—12½ μ. Wall ½—1 μ broad.

Several cases, all childern at the age of 6 till 8 years, many of them from orphanages. All have good recognizable patches in the hair of the scalp. Furthermore there is no difference in the susceptibility of the different races, as Javanese, Chinese, Eurasian and European childern are all affected.

Clinical diagnosis, Microsporia.

Ectotrichophyton CASTELLANI et CHALMERS Man. Trop. Med. ed. 3, 1003—1008, 1919.

Microtrichophyton NEVEU LEMAIRE Précis Parasitol. Hum. 53—54, 1921.
Ateleothylax OTA et LANGERON Ann. Parasitol. Hum. Comp. 1. 333. 1923.
Matruchotiella GRIGORAKIS C. R. Acad. Sc. Paris 179. 1424. 1924.
Spiralia GRIGORAKIS Ann. Sc. Nat. Bot. X. 7. 409. 1925.
Chlamydo aleurosporia GRIGORAKIS Ann. Sc. Nat. Bot. X. 7. 412. 1925 pro parte-
Clostero-aleurosporia GRIGORAKIS Ann. Sc. Nat. Bot. X. 7. 415. 1925 pro parte.

Colonies large, velvety till powdery, with central boss and sometimes some irregular folds. Border often with radiating rays. Growth rapid. Mycelium with abundant formation of spiral-hyphae. The spirals originating from broad hyphae with often granular walls. The so-called modular organs represent degenerated ascogonia. Aleuriospores abundant. Closterospores scanty, small, few-celled, smooth, and thinwalled.

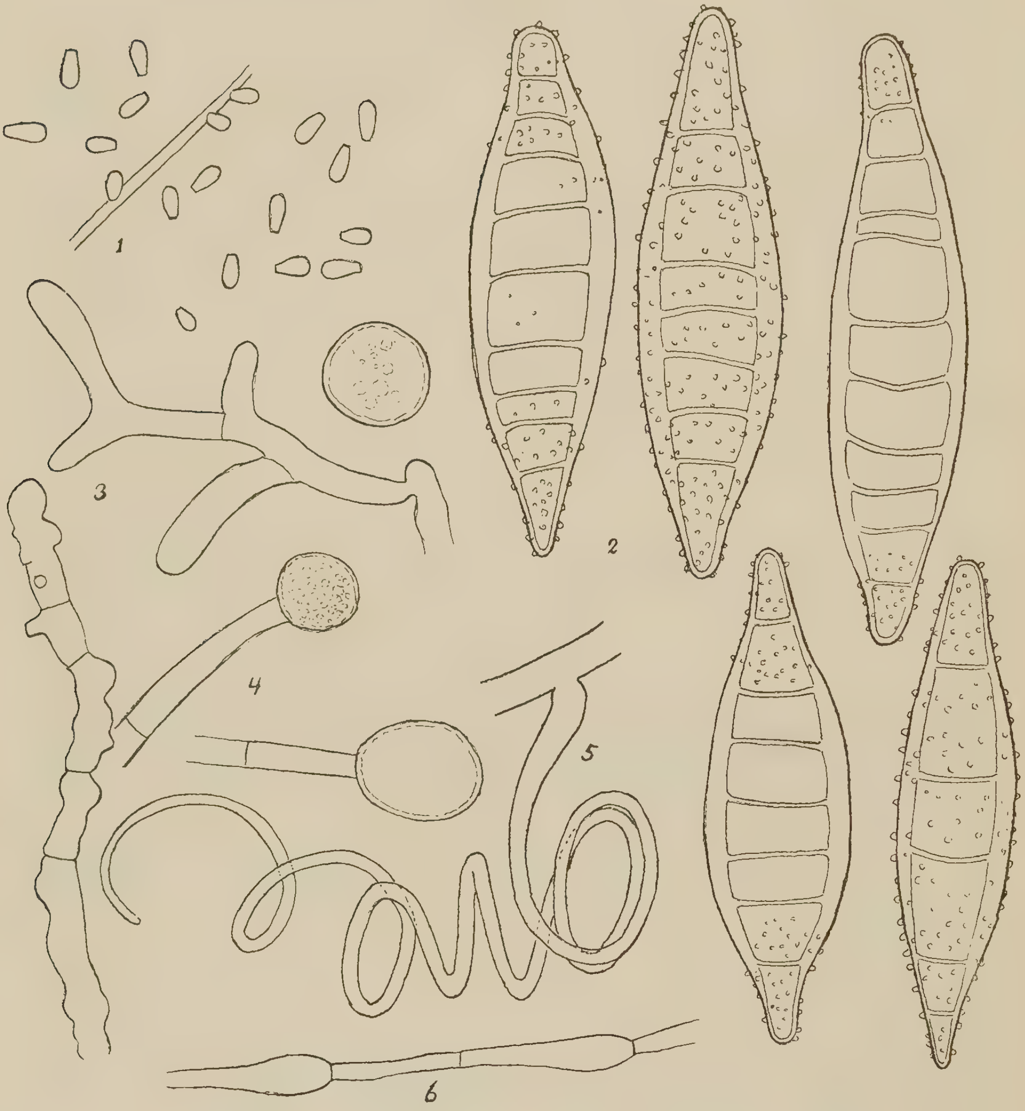

Fig. 1. *Microsporum felineum.*
1. Aleuriospores; 2. Closterospores; 3. Hyphae; 4. Chlamydospores; 5. Spiral hypha; 6. „Mycelium en raquette".

Chlamydospores present.

Type species, ECTOTRICHOPHYTON MENTAGROPHYTES (ROBIN) CASTELLANI et CHALMERS.

ECTOTRICHOPHYTON MENTAGROPHYTES (ROBIN) CASTELLANI et CHALMERS Man. Trop. Med. ed. 3, 1005. 1919.

Microsporon mentagrophytes ROBIN Hist. Nat. Vég. Paras. qui croissent sur l'homme. Anim. 430—436. 1853.

Sporotrichum (*Microsporum*) *mentagrophytes* SACCARDO Syll. Fung. 4, 100, 1886.

Trichophyton mentagrophytes GEDOELST Champ. Paras. Homme 86, 87, 1902.

Trichophyton gypseum BODIN Champ. Paras. Homme 115—118, 1902.

Trichophyton asteroides SABOURAUD Maladies du cuir chevelu 3, 347, 1910.

Trichophyton radiolatum SABOURAUD Maladies du cuir chevelu 3, 355—356, 617—621, 1910.

Trichophyton mentagrophytes var. *asteroides* Neveu Lemaire Précis Parasitol. Anim. Domest. 69—71, 1912.

Ectotrichophyton (*Microtrichophyton*) *radiolatum* CASTELLANI et CHALMERS Man. Trop. Med. ed. 3, 1006, 1919.

Sabouraudites asteroides OTA et LANGERON Ann. Parasitol. Hum. Comp. 1, 327, 328. 1923.

Aleurosporia rosacea GRIGORAKIS Ann. Sc. Nat. Bot X 7, 413, 1925.

Microsporum (*Closterosporia*) *asteroides* GUIART et GRIGORAKIS Lyon Méd. 141, 377, 1928.

Microsporum (*Closterosporia*) *radiolatum* GUIART et GRIGORAKIS Lyon Méd. 141, 377, 1928.

Trichophyton gypseum var. *radiolatum* BRUHNS et ALEXANDER in JADASSOHN, Handb. d. Haut u. Geslechtskrankh. 11, 175—176, 1928.

Ctenomyces mentagrophytes LANGERON et MILOCHEVITCH, Ann. Parasitol. Hum. Comp. 8, 484, 1930.

Spiralia mentagrophytes GRIGORAKIS C. R. Soc. Biol. 109, 186—188, 1932.

Ectotrichophyton mentagrophytes var. *radiolatum* (BRUHNS et ALEYANDER) DODGE, Med. Mycol. 505, 1935.

Rapid growing colonies, rather flat, with central boss and sometimes some indistinct furrows. Margin weakly radiating; surface granular, strikingly powdery. Colour at first white, afterwards pale cream colour about light buff. Part of colony submerged, till 5 mm in the medium. On the reverse colour dark redbrown, about between liver brown and Carob brown. Mycelium branched; at first thin and sparingly septated, afterwards much broader and richly septated. The separate threads 1—6 μ broad. Some threads undulating, other thick hyphae with swellings near the septae. Near the surface of the colony the mycelium shows many side-branches originating nearly under right angles. Those threads 2—3½ μ broad, often with granular cellwalls. On those branches the spiral-hyphae are born, which are 1½—2 μ broad and provided with 1—20 coils. Those coils are dense or more or less stretched. On the thin hyphae the aleuriospores are formed laterally. They are colourless, subglobose, born on a very small denticulation, 2½—3½ μ in diam. or 3—3½ × 2—3 μ.

Closterospores mostly terminal, scanty, small, clubshaped, straight or curved, thinwalled, smooth, with 3—5 mostly 3 crosswalls, rounded top and attenuated near the truncated base.

They are 20—26 μ long, 5—8 μ broad near the top. Chlamydospores terminal, lateral or intercalary, globose till subglobose, thickwalled, 13—20 μ in diam., wall ½—1 μ broad.

The mycelium at the surface of and especially in the medium, forms long rows of rounded cells 3—7 μ in diam. or 5—8 × 2½—5 μ.

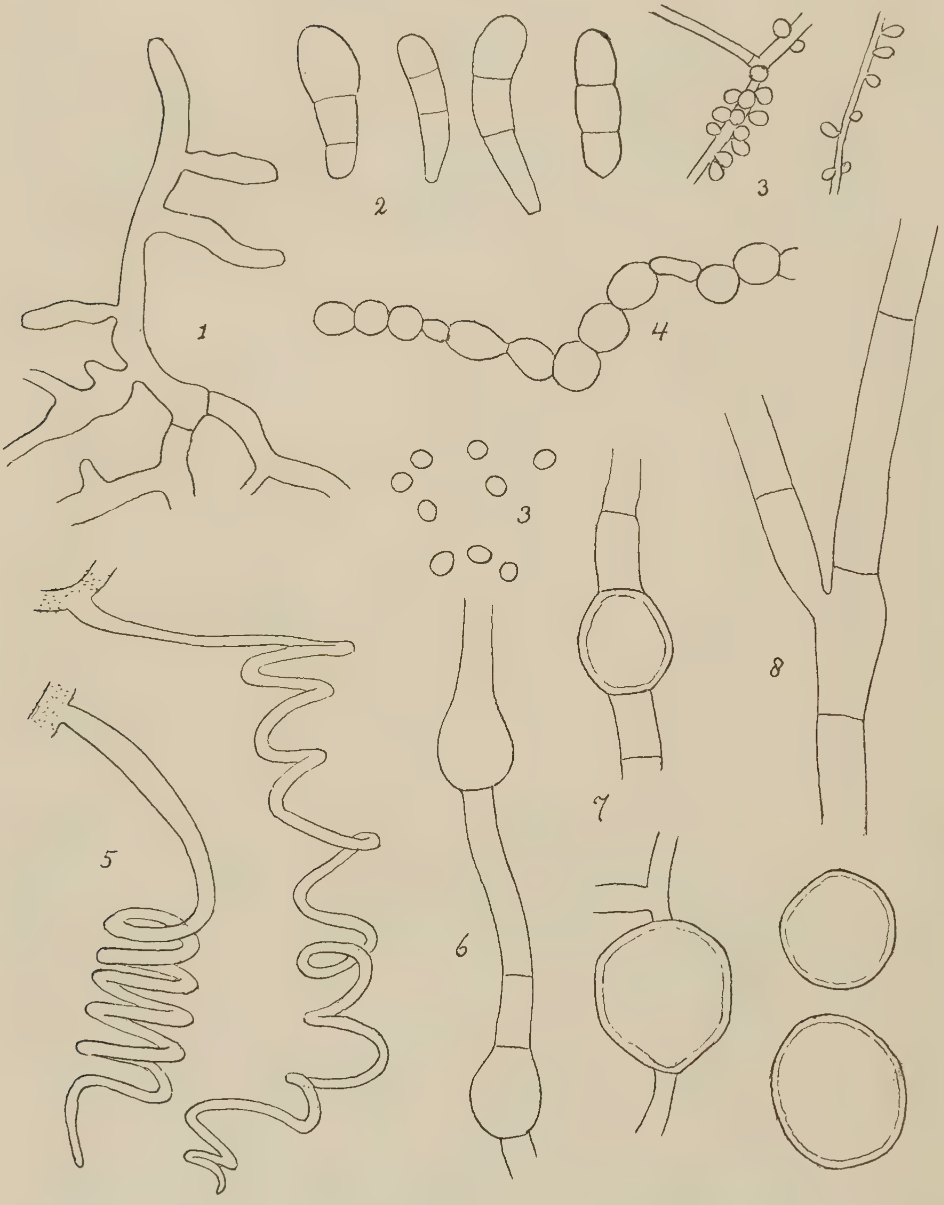

Fig. 2. *Ectotrichophyton mentagrophytes.*
1 Mycelium of the surface of the colony; 2. Closterospores; 3. Aleuriospores; 4. Mycelium from the bottom of the colony; 5. Spiral hyphae; 6. ,,Mycelium en raquette"; 7. Chlamydospores; 8. Common mycelial thread.

European man with infection between the toes of the right feet.
Chinese man with patches on belly and sides of the trunk.
Clinical diagnosis: Epidermophytia.

Sabouraudiella BOEDIJN nov. gen.

Culturis effusis, centro elevato, velutinus vel farinaceis, radiato-sulcatis, saepe coloris diffundentibus. Aleuriosporae copiosae.

Closteroporae magnae, cylindraceae, multizeptatae, leves, tenuiter tunicatae, Chlamydosporae globosae, crasse tunicatae.

Colonies velvety till more or less powdery, with elevated centre and with or nearly without radial grooves. Often a colour is formed, which is partially diffusing into the medium. Growth rather rapid. Aleuriospores abundant. Closterospores large, cylindrical, many septated, smooth and thinwalled. In some species not frequent and only in the primary culture or on special media. Chlamydospores abundant, especially in old cultures.
Type species: SABOURAUDIELLA PURPUREA (BANG) BOEDIJN.

Sabouraudiella purpurea (BANG) BOEDIJN nov. comb.
Trichophyton purpureum BANG Ann. Derm. Syphilogr. V 1, 234—238, 1910.
Trichophyton persicolor SABOURAUD Maladies du cuir chevelu 3, 371—374, 1910.
Trichophyton rubidum PRIESTLEY Med. Journ. Australia 4, 2, 474, 1917.
Ectotrichophyton (*Microtrichophyton*) *persicolor* CASTELLANI et CHALMERS Man. Trop. Med. ed. 3, 1005—1006, 1919.
Epidermophyton salmoneum FROILANO DE MELLO C. R. Soc. Biol. 84, 239—240, 1921.
Sabouraudites (*Aleuramma*) *persicolor* OTA et LANGERON Ann. Parasitol. Hum. Comp. 1, 329, 1923.
Epidermophyton plurizoniforme MAC CARTHY Ann. Derm. Syphiligr. VI, 6, 31—37, 1925.
Epidermophyton lanorosum MAC CARTHY Ann. Derm. Syphiligr. VI, 6, 49—53, 1925.
Sabouraudites plurizoniformis BRUMPT Précis Parasitol. ed. 4, 1291, 1927.
Microsporum (*Clostervaleurosporia*) *persicolor* GUIART et GRIGORAKIS Lyon Méd. 141, 377, 1928.
Epidermophyton purpureum (BANG) DODGE Med. Mycol. 485, 1935.
Epidermophyton rubidum (PRIESTLEY) DODGE Med. Mycol. 486, 1935.
Epidermophyton persicolor (SABOURAUD) DODGE Med. Mycol. 486, 1935.

Colonies at first rather flat, with central boss, afterwards with irregular folds, elevated centre and some radial grooves near the periphery. Surface velvety till powdery. Colour at first white, afterwards center pink, changing gradually from light Congo pink till light Corinthian red. Margin nearly white. On the reverse colour dark red, about between ox blood and Garnet brown, diffusing into the medium. Mycelium richly branched and septated; some cells with dark red droplets. The separate threads 1—5½ μ broad. Aleuriospores abundant, sessile, born laterally on the thin hyphae. They are broadly clavate with rounded top and truncated base 4—9 × 1½—5 μ. Closterospores especially in the primary cultures, terminal or intercalary, long cylindrical, with truncated base, gradually increasing in breadth near the rounded top. Except for the swollen tops hardley broader than the supporting hyphae. Very variable as to shape and dimensions, straight or curved, thinwalled and smooth, with 1—6 mostly 3—4 crosswalls, 15—58 μ long, 3½—6 μ broad near the top, 2—3 μ broad near the base. Most common dimensions 28—43 × 4—5 μ. Those born intercalary sometimes with a threadlike appendage. Chlamydospores common.

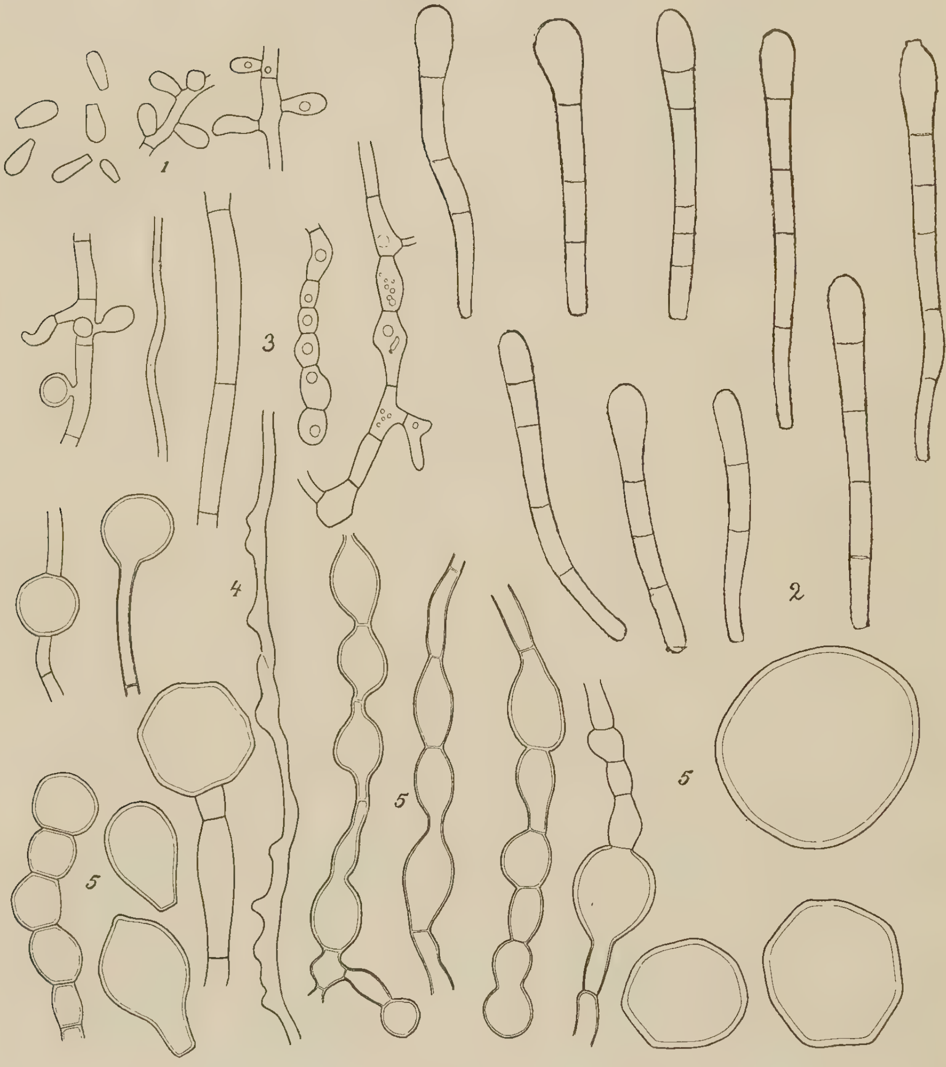

Fig. 3. *Sabouraudiella purpurea.*
1. Aleuriospores; 2. Closterospores; 3. Hyphae, some with red droplets; 4. Pectinate hypha; 5. Formation of chlamydospores and ripe chlamydospores.

They are born lateral, terminal and especially intercalary; often arranged in chains; globose till susglobose or somewhat anguiar, 10—30 μ in diam., cellwall up till 1 μ or slightly broader.

By far the most common dermatophyte in our region. It chiefly attacks the glabrous skin and is found on all races. Sometimes nearly the whole body is affected.

Clinical diagnosis: Epidermophytia.

In the literature this species is often mentioned under the name *Trichophyton rubrum*. This however is a very different species as can be seen from our description.

Sabouraudiella rubra (CASTELLANI) BOEDIJN nov. comb.
Epidermophyton rubrum CASTELLANI Brit. Journ. Derm. Syphilis 22, 147—151, 1910.
Trichophyton rubrum SEMON Brit. Journ. Derm. Syphilis 34, 398, 1922.
Sabouraudites ruber OTA et LANGERON Ann. Parasitol. Hum. Comp. 1, 328, 1923.

Colonies flat conical up till 3 mm high in the centre. Surface cerebriform, the folds gradually radially arranged near the periphery. Culture up till 4 mm submerged into the medium. At first powdery, afterwards the whole colony takes a more or less waxy appearance. Coulour in the centre a dark red, about deep livid brown, vinaceous brown, dark vinaceous brown till dark vinaceous drab. The rest of the colony brownish drab, light brownish drab till vinaceous drab. On the reverse the colour is Eugenia red, Acajou red till burnt lake. Mycelium richly branched and septated, the separate threads 1½—5 μ broad.

Aleuriospores numerous, sessile, born laterally on the hyphae. They are colourless, broadly clavate, with rounded top and truncated base, 3½—6 × 2½—3 μ.

Closterospores abundant, originating laterally is terminally seldom intercalary on the mycelial threads. They are long cylindrical, straight, seldom curved, thinwalled, the poles broadly rounded; with 3—9 crosswalls, 15—90 × 3—8 μ. Most common dimensions 57—72 × 5—6½ μ. Sometimes provided with a threadlike appendage 13—77 × 2½—3½ μ.

Chlaneydospores abundant, especially in old cultures, lateral, terminal but mostly intercalary on the hyphae. Often forming long chains.

Mature chlamydospores globose or weakly angular, 15—21 μ in diam. Cellwall 1—2 μ broad.

Javanese man with patches on right hand.

Malayan man with patches on right leg, tighs, mons pubis and left hand.

Clinical diagnosis: Epidermophytia.

A very striking species, much remote from *S. purpurea*. Not only the giantcolony, but also shape and dimensions of the closterospores are wholly different. It is a rare form, only met twice in Djakarta. The species conception is in accordance with those of DODGE (9) and especially of MUSKATBLIT (17) who made a careful comparative study of both species.

Sabouraudiella interdigitale (PRIESTLEY) BOEDIJN nov. comb.
Trichophyton interdigitale PRIESTLEY Med. Journ. Australia 4, 2, 495, 1917.
Sabouraudites interdigitalis OTA et LANGERON Ann. Parasitol. Hum. Comp. 1, 328, 1923.
Epidermophyton interdigitale (PRIESTLEY) MAC CARTHY Ann. Derm. Syphiligr. VI, 6, 24, 1925.
Epidermophyton variabile KARRENBERG Arch. Derm. Syphilol. 17, 519—532, 1928.

Colonies very swift growing, without noticeable structures; sometimes with an indistinct boss in the centre. Surface velvety colour pure white, in old cultures sometimes slightly tinged with buff near the margin. Mycelium branched and septated, the separate threads 1—9 μ broad.

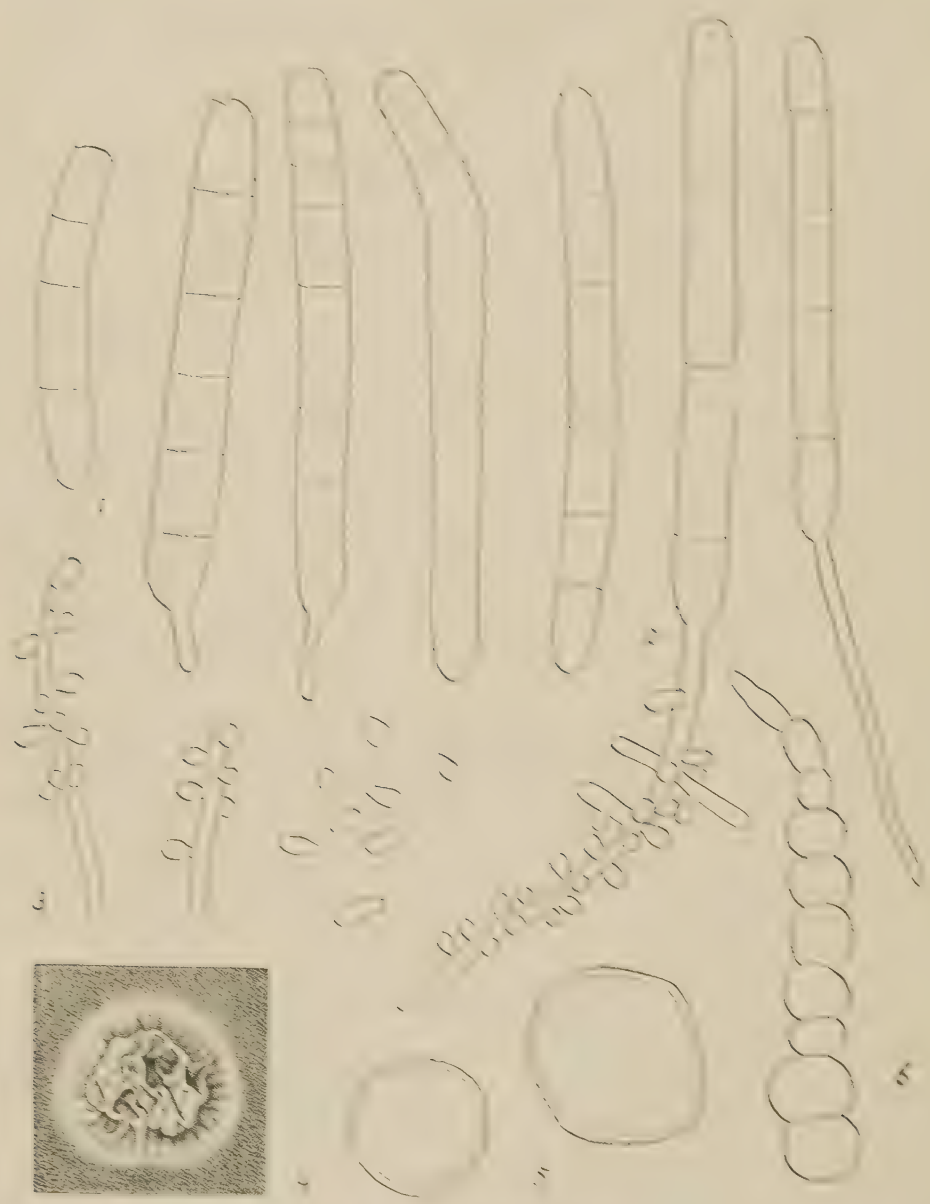

Fig. 4. *Sabouraudites ruber*

1. Closterospores; 2. Closterospore with formation of aleuriospores at the appendage; 3. Aleuriospores; 4. Giant colony; 5. Chlamydospores; 6. Hypha with swollen cells.

Aleuriospores abundant, lateral, sessile, single or in short chains from 2 till 2 spores, globose to sligtly elongated, 2—5 μ in diam.

Closterospores formed on the thicker hyphae; terminal, lateral and especially intercalary. Sometimes the intercalary closterospores are separated from each other by thin hyphal portions. After detachment they may show pedicels on one or both poles or even two spores may be connected. When on a place of tranching spore formation takes place, a spore with another dorsally attached may originate, or the pedicel may be branched. They are cylindric till long elliptical, straight or curved, thinwalled; one of the poles mostly stronger attenuated than the other, with 2—7 mostly 5 crosswalls and often with 1 or 2 pedicels or without appendages, 26—70 × 6—13 μ. Most common dimensions 50—60 × 8—10 μ. Pedicel 6—87 × 1—3½ μ, very rarely septated. Chlamydospores terminal, lateral or intercalary, globose, 9—16 μ in diam. Cellwall about 1 μ broad.

European man with patches on face and right hand.
Clinical diagnosis: Epidermophytia.

Epidermophyton SABOURAUD Les Maladies du cuir chevelu 3, 420—466, 1910.

Colonies velvety, with elevated centre and radial furrows. Slow growing. No aleuriospores ever formed. Closterospores cylindrical, thinwalled, smooth, septated born single or often in groups on the hyphae. Chlamydospores present.

Type species: EPIDERMOPHYTON FLOCCOSUM (HARZ) LANGERON et MILOCHEVITCH.

EPIDERMOPHYTON FLOCCOSUM (HARZ) LANGERON et MILOCHEVITCH. Ann. Parasitol. Hum. Comp. 8, 495—497, 1930.
Acrothecium floccosum HARZ Bull Soc. Imp. Nat. Moscou 44, 124, 1871.
Blastotrichum floccosum BERLESE et VOGLINO in SACCARDO Syll. Fung. 4 Add. 376, 1886.
Trichophyton intertriginis Sabouraud Dermatol. Topograph. 300, 1905.
Trichophyton inguinale SABOURAUD Arch. Méd. Expérim. Anat. Path. 19, 565—586, 737—766, 1907.
Trichophyton cruris CASTELLANI Journ. Trop. Med. Hyg. 11, 262, 1908.
Epidermophyton inguinale SABOURAUD Maladies du cuir chevelu 3, 420—444, 1910.
Epidermophyton cruris CASTELLANI et CHALMERS Man. Trop. Med. 609—610, 1910.
Epidermophyton plicarum NICOLAU Ann. Derm. Syphiligr. V, 4, 65—87, 1913.
Dactylium? floccosum SARTORY Champ. Paras. Homme Anim. 871, 1923.
Closterosporia inguinalis GRIGORAKIS Ann. Sc. Nat. Bot. X, 7, 411, 1925.
Epidermophyton clypeiforme MAC CARTHY Ann. Derm. Syphiligr. VI, 6, 27—30, 1925.
Microsporum (*Closterosporia*) *inguinale* GUIART et Grigorakis Lyon Méd. 141, 377, 1928.
Fusoma cruris VUILLEMIN C. R. Acad. Sc. 189, 405, 1929.
Epidermophyton floccosum var. *clypeiforme* (MAC CARTHY) DODGE Med. Mycol. 483, 1935.

Colonies slow growing, with irregular radial furrows and elevated centre, sometimes umbilicate. Surface dry, powdery. Colour greenisth gray, about smoke grey or light grayish olive, but with distinct greenish hue.

Mycelium branched and septated, the separate threads 1—6 μ broad. Aleuriospores never present. Closterospores abundant, mostly lateral or ter-

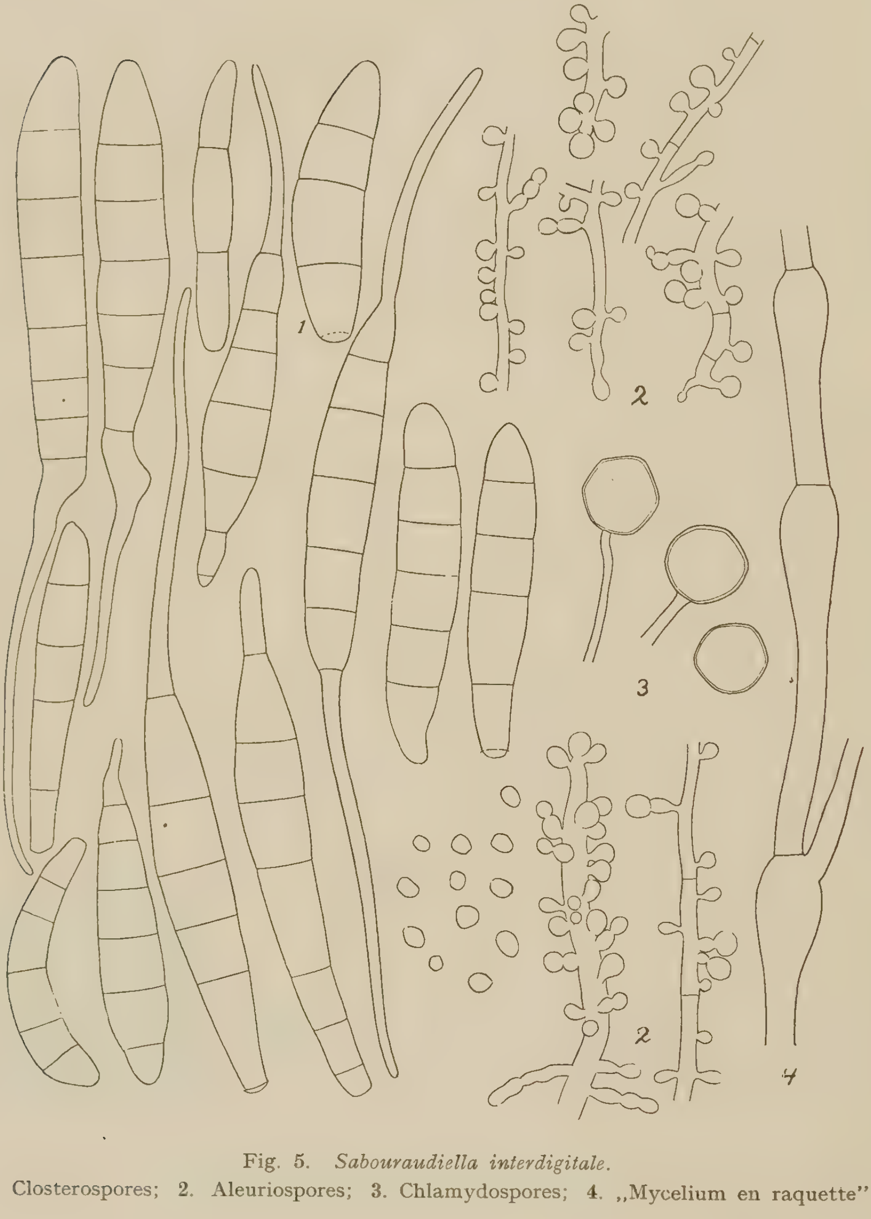

Fig. 5. *Sabouraudiella interdigitale.*
1. Closterospores; 2. Aleuriospores; 3. Chlamydospores; 4. „Mycelium en raquette".

minal, single or in groups of 2 till 6. They are cylindrical, thinwalled with rounded top and slightly attenuated near the truncated base, with 2—5 mostly 3 crosswalls, 23—43 × 7—10 μ, most common dimensions 27—30 × 8μ Chlamydospores numerous, especially in old cultures. They are originating lateral, terminal or intercalary on the hyphae and are globose 16—24 μ in diam. Cellwall 1—2 μ broad.

Malayan man with patches in the inguinocrural folds.
Clinical diagnosis: Epidermophytia.

Epidermophyton angustisporum BOEDIJN nov. spec.

Culturis crateriformibus, radiato-sulcatis, primo velutinis deinde farinaceis, dilute ochraceis, Mycelium e hyphis hyalinis 1½—7 μ crassis, septatis, ramosis compositum.

Closterosporae terminales, cylindraceae, leves, tenuiter tunicatae, basi attenuatae 1—7 plerumque 3—5 septatae, 19—80 μ longae, apice 3—5 μ et basi 2—2½ μ crassae.

Chlamydosporae globosae vel elongatae, 9—15 μ in diam. vel 12—18 × 9—17 μ.

Colonies nearly crateriform, strongly radially plicate, up till 5 mm high. Surface at first short fluffy, afterwards more or less powdery. Colour at first white, afterwards pale ocher about between light buff and cream color. On the reverse colour dark brown, about warm sepia. Mycelium branched and septated, the separate threads 1½—7 μ broad.

Aleuriospores never formed. Closterospores numerous, mostly originating terminally on the hyphae. They are cylindrical with rounded top, gradually attenuated near the truncated base; mostly straight, sometimes curved, thinwalled with 1—7 mostly 3—5 croswalls, 19—80 μ long, 3—5 μ broad near the top, 2—2½ μ near the base. Most common dimensions 35—40 μ long, 3½—4 μ broad near the top, 2 μ near the base. Chlamydospores mostly intercalary, sometimes in short chains, globose till elongated 9—15 μ in diam. or 12—18 × 9—17 μ. Cellwall up till 1 μ broad.

Arab girl with scaling patches round the mouth, on the nose, trunk and extremities.
Clinical diagnosis: Tinea imbricata.
But the scales were not so regularly arranged and the isolation from them yielded the above mentioned species.

Trichophyton MALMSTEN Trichophyton tonsurans härskärande Mögel, 1845.
Neotrichophyton CASTELLANI et CHALMERS Man. Trop. Med. ed. 3, 1002, 1919.
Atrichophyton CASTELLANI et CHALMERS Man. Trop. Med. ed. 3, 1002, 1919.
Megatrichophyton NEVEU LEMAIRE Précis Parasitol. Hum. 54, 1921.
Aleurosporia GRIGORAKIS Ann. Sc. Nat. Bot. X, 7, 413, 1925.

Colonies more or less crateriform or with central button, powdery and often furrowed. Mycelium with abundant aleuriospores born laterally on the hyphae. Closterospores never formed. Chlamydospores present.

Type species: TRICHOPHYTON TONSURANS MALMSTEN.

TRICHOPHYTON PLICATILE SABOURAUD Maladies du cuir chevelu 3, 330—333, 1910.
Neotrichophyton plicatile CASTELLANI et CHALMERS Man. Trop. Med. ed. 3, 1002, 1919.
Aleurosporia plicatilis GRIGORAKIS Ann. Sc. Nat. Bot. X, 7, 413, 1925.

Colonies with elevated centre, which is cerebriform by irregular folds, margin with some radial furrows. In old cultures some of the folds cracking. Surface powdery and of a white colour. Mycelium much branched and septated,

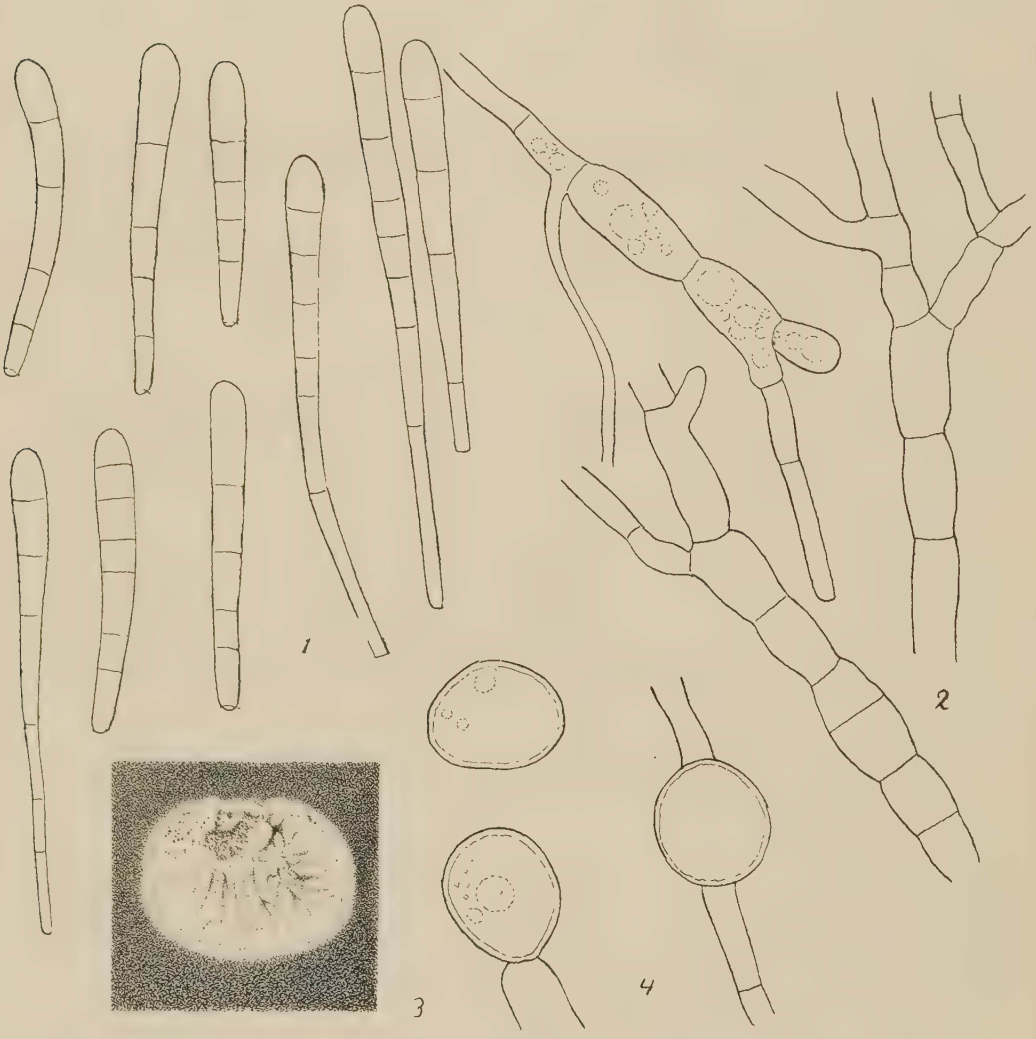

Fig. 6. *Epidermophyton angustisporum.*
1. Closterospores; 2. Mycelium; 3. Giant colony; 4. Chlamydospores.

the separate threads 1—7 μ broad. Aleuriospores abundant, laterally born on the hyphae; sessile, broadly clavate with truncated base and rounded top, 4—11 × 2—6 μ. Chlamydospores terminal and intercalary, globose with only slightly thickened cellwall, 10—16 μ in diam. In aging the cells of the hyphae are swelling, giving rise to long chains of globose till elongated cells, which at last are braking apart. So in old cultures large numbers of spore-like cells are to be found.

Soudanese man with patches on trunk and extremities.
Clinical diagnosis: Epidermophytia.

Achorion Remak Diagnostische u. pathogen. Unters, 193, 1845.
Schoenleinium Johan Olsen Centralbl. Bakt. II, 3, 276—284, 1897.
Lepidophyton Tribondeau Arch. Med. Navale 72, 5—52, 1899.
Endodermophyton Castellani et Chalmers Man. Trop. Med. 610, 1910.
Favotrichophyton Neveu Lemaire Précis Parasitol. Hum. 55, 1921 pro parte.
Bodinia Ota et Langeron Ann. Parasitol. Hum. Comp. 1, 329, 1923.
Grubyella Ota et Langeron Ann. Parasitol. Hum. Comp. 1, 330, 1923.
Arthrosporia Grigorakis Ann. Sc. Nat. Bot. X, 7, 414, 1925 pro parte.

Colonies slow growing, heaped, compact, more or less hemisphaerical, cerebriform; at first sometimes with very short velvet, afterwards smooth, often humid or waxy in appearance. Mycelium branched and septated. Mostly only chlamydospores present.

Type species: Achorion Schoenleini (Lebert) Remak.

Achorion concentricum (Blanchard) Guiart et Grigorakis. Lyon Méd. 141, 377, 1928.
Trichophyton concentricum Blanchard in Bouchard Traité Path. Gén. 2, 916—919, 1896.
Lepidophyton concentricum Gedoelst Champ. Paras. Homme Anim. Domest. 147—149, 1902.
Aspergillus lepidophyton Pinay Bull. Inst. Pasteur 1, 761—774, 1903.
Aspergillus Tokelau Wehmer Centralbl. Bakt. I, 35, 140, 1903.
Trichophyton Mansoni Castellani Brit. Med. Journ. 2, 1277—1279, 1905.
Aspergillus concentricum Castellani Trans. Intern. Derm. Cong. 6, 667—671, 1907.
Endodermophyton Castellani Perry Ceylon Med. Rept. 1907.
Endodermophyton concentricum (Blanchard) Castellani et Chalmers Man. Trop. Med. 610, 1910.
Endodermophyton indicum Castellani Journ. Trop. Med. Hyg. 14, 81—83, 146, 1911.
Oospora concentrica Hanawa et Nagai Jap. Zeitschr. Derm. Urol. Ergänzung. Band 47, 84, 1917.
Endodermophyton tropicale Castellani et Chalmers Man. Trop. Med. ed. 3, 1017—1020, 1919.
Arthrosporia indica Grigorakis Ann. Sc. Nat. Bot. X, 7, 414, 1925.
Arthrosporia tropicalis Grigorakis Ann. Sc. Nat. Bot. X, 7, 414, 1925.
Endodermophyton Mansoni Brumpt Précis Parasitol. ed. 4, 1299, 1927.
Achorion (Endodermophyton) indicum Guiart et Grigorakis Lyon Méd. 141, 377, 1928.
Mycoderma concentricum Vuillemin C. R. Acad. Sci. 189, 405, 1929.

Colonies slow growing, convex, up till 13—15 mm high in centre and up till 3 mm submerged into the medium. Surface cerebriform, near the periphery furrows more or less radially arranged. At first covered with very short velvet, afterwards more or less glabrous, centre sometimes humid. Colour at first dull

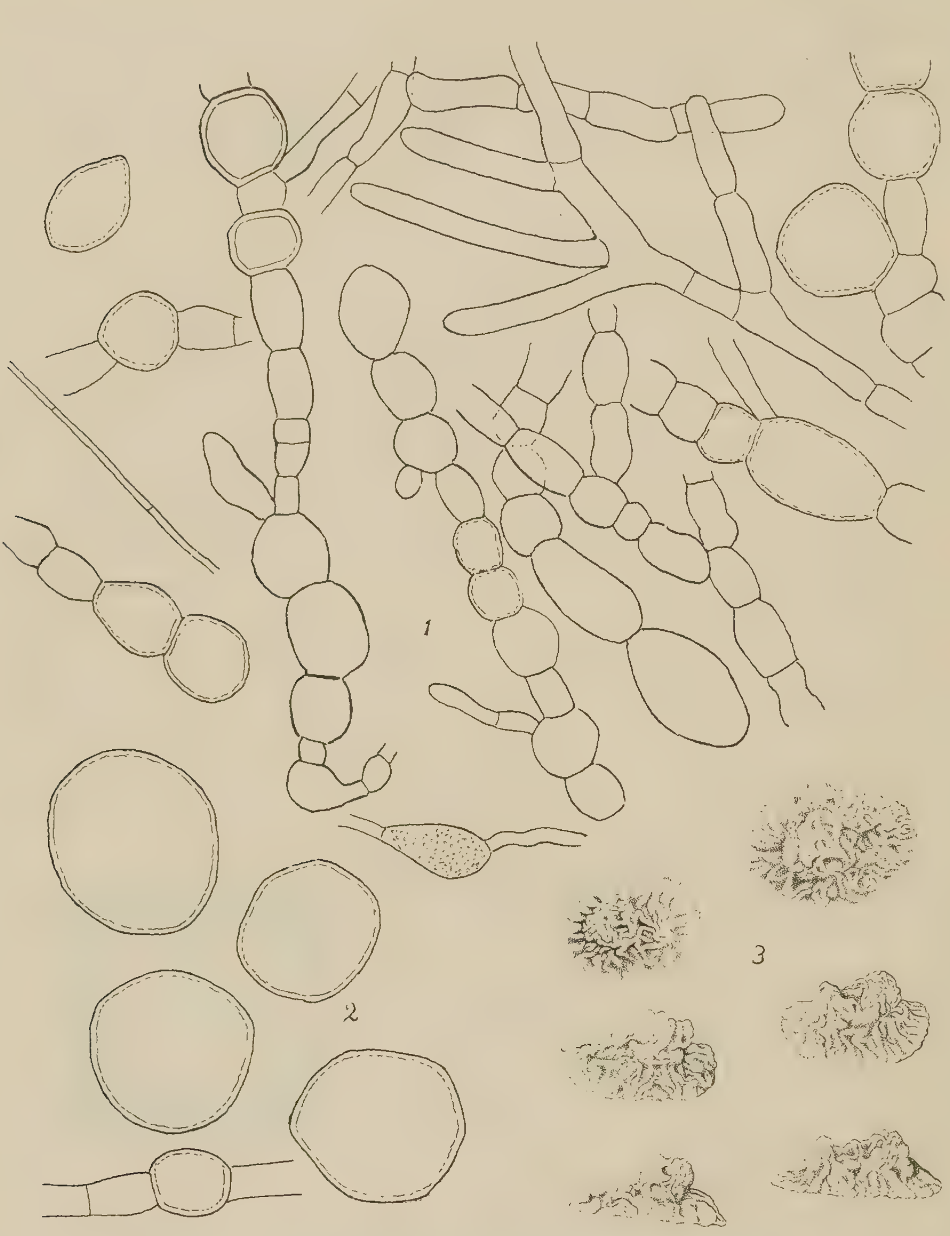

Fig. 7. *Achorion concentricum.*
1. Mycelium and chlamydospore-formation; 2. Ripe chlamydospores;
3. Giant colonies, in face and side view.

orange, about zinc orange or between zinc orange and ochraceous orange, afterwards centre a dirty ocher, about pinkish buff, tilleul buff till avellaneous. Rest of colony nearly white or between pinkish buff and avellaneous. In old cultures the whole colony a dirty brown, about. Natal brown till Clove brown, with a very short grey tomentum round the border.

Mycelium intricately intertwined, especially in old cultures, richly branched and septated. The separate threads 1—6 μ broad. The older hyphae often more or less constricted at the septae, afterwards giving rise to torulose threads with cells 3—6 μ in diam. or 5—11 × 3—5 μ.

Chlamydospores numerous; terminally or intercalary on the hyphae, globose or somewhat angular 8—16 μ in diam. Most common dimensions 12—14 μ in diam. Cellwall 1—1½ μ broad.

(In a primary culture a small number of aleuriospores could be observed. They are elliptic with truncated base, 3—6 × 2—3 μ, sessile and born laterally on the hyphae.)

A Dajak man, two Malayan men and one Malayan woman. In all cases nearly the whole body was affected.

Clinical diagnosis: Tinea imbricata.

ACHORION OCHRACEUM (SABOURAUD) GUIART et GRIGORAKIS. Lyon Méd. 141, 377, 1928.
Trichophyton ochraceum Sabouraud Ann. Derm. Syphiligr. IV, 9, 628—834, 1908.
Ectotrichophyton ochraceum CASTELLANI et CHALMERS Man. Trop. Med. ed. 3, 1004, 1919.
Favotrichophyton ochraveum (SABOURAUD) NEVEU LEMAIRE Précis Parasitol. Hum. 55, 1921.
Grubyella ochracea OTA et LANGERON Ann. Parasitol. Hum. Comp. 1, 330, 1923.
Arthrosporia ochracea GRIGORAKIS Ann. Sc. Nat. Bot. X, 7, 414, 1925.

Colonies slow growing, hemisphaerical, cerebriform, with glabrous, humid surface. Colour dirty ochraceous, about pinkish buff but mostly more grey. Mycelium intricately branched, richly septated. The separate threads 2—4 μ broad, often undulating, anastomosing and constricted in some places. Only chlamydospores present. They are globose till elongated 6—16 μ in diam. or 10—14 × 7—12 μ and born laterally, terminally or intercalary on the hyphae.

A boy, Minangkabau with a small patch in the right axilla.

Clinical diagnosis: Trichophytia.

Achorion decipiens (BOEDIJN et VERBUNT) BOEDIJN nov. comb.
Favotrichophyton decipiens BOEDIJN et VERBUNT Mycopathologia 1, 194, 1938.

Colonies slow growing, consisting of a hemisphaerical central portion with cerebriform surface, surrounded by a very broad and flat margin, partly submerged in the medium and feathery branching at the periphery.

For the naked eye the colony is smooth, but finely pubescent under a handlens. Colour of centre greyis, about light drab, margin dirty violet brown, about light cinnamon drab till cinnamon drab. On the reverse the colour is dirty reddish. Mycelium richly branched and septated; the separate threads often undulating and 1½—7 μ broad. Cells often provided with oildrops, hyaline or with a dirty orange colour in the older parts of the colony. Only chlamydospores present; anundant, terminal, lateral or intercalary. At first globose, when fully ripe, typical oval till elliptic, with thickened cellwall, 17½—32 × 12—20 μ.

Most common dimensions 20—24 × 14—17 μ.

Cellwall up till 1 μ broad. The wall which cuts of the chlamydospore from the supporting hyphae is mostly thin.

Two Eurasian boys, one 8 the other 10 years old, with typical patches in the hair of the scalp. Clinical diagnosis: Microsporia.

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A neglected definition of the genus *Cryptococcus* made by Rivolta (1873)

by

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(28.5.1951)

In spite of the fact that, since the year 1925, the writer demonstrated the untenability of the genus *Cryptococcus* Kütz. as a "medical" genus, and the inconsistency of the generic name, it is evident a recent tendency of a number of american, medical mycologists, to reconvalidate this generic name, apparently under the suggestion of SKINNER (1950).

The definition of *Cryptococcus* as a "medical" genus is commonly attributed to the french mycologist VUILLEMIN (1901). Really, the first attempt is that of ROBIN (1853), shortly followed by the fair more complete definition by RIVOLTA, the discoverer of the etiological agent of the epizootic lymphangitis of Solipeds: *Cryptococcus farciminosus* Riv. et Mic., now *Histoplasma farciminosus* (Riv. et Mic.) Cif. et Red.

ROBIN, 1847 (pag. 47), described the "champignon du tube digestif du Lapin et autres herbivores" of REMACK (1845) under the binomial *Cryptococcus guttulatus,* in the treatise of 1853 (pag. 327) catalogued under the genus *Cryptococcus,* with *C. cerevisiae* (Turpin) Kütz. as well as *C. guttulatus* Robin. Also in this most complete second edition, ROBIN indicated only (a few) morphological characteristics of both species, mixing the yeasts found on beer together with species found on men and animals.

In the frequently quoted, but rarely consulted book of the year 1873, RIVOLTA defined the genus *Cryptococcus* also on the base of the fermentative characteristics and for the "habitat" on men and animals, anticipating the VUILLEMIN amendement. Species of this genus are quoted many times, but the genus is more clearly and diffusely defined in the pages 414—415. A free translation of the paragraphs dedicated to *Cryptococcus* is: "The Cryptococca "are very small cells, reproducing by buds developing at one end. A number "of pathologists and mycologists considered *Cryptococcus* identical to *Saccha-"romyces* or *Cryptococcus cerevisiae,* but really it is distinct because it does not "produce an alcoholic fermentation, but it is a decomposing agent of the "substrata . . . Simple Cryptococca were observed by a number of Authors . . ." (here seven names of students of the epoch, five forms of "catarrh" and the pseudodyphtheric membrane on man are quoted. Therefore, according to the opinion of HALLIER on the "morpha", an exageration of TULASNE theory of pleomorphism, *Oidium albicans* is supposed to derivate from the micrococca of the pseudodyphtheric membranes, through a cryptococcic stage). "On the "diabetic urines [the Cryptococca] is developing in 24 hours, forming a pellicle "on the surface, and even on the urine without sugar" (two Authors quoted) "(pag. 215). "In the animals, the writer observed Cryptococca in the pus of "farcinics abscesses and in the ventricle of hen dead for an epizootic morbus. "Avoiding confusion, it is preferable to indicate this vegetable with the name "*Cryptococcus* and with the anatomical region or disease or body fluid in which "is found. In my opinion [such Cryptococca] are not to be confused with "*Cryptococcus cerevisiae*, as the differences are sufficiently conspicuous ..." (In the next paragraph is quoted *Cryptococcus clava* Wedl, only according to the description of Wedl, now an unrecognizable Hyphales found in the stomach, chiefly in the renning ventricle of the Ruminants).

In addition to the above reported quotations, in this volume are described or recorded the following Cryptococca [1]):

1) *Cryptococcus* afterward named *farciminosus* Riv. et Mic. (pag. 103, 246 et seqq., 524, 525);

2) Cryptococca of the psoriasis (fig. 174 and pag. 469), from a personal case; a yeast-like form;

3) Cryptococca from keratophyllic disease of the horse foot, a yeast-like form (pag. 525, fig. 153);

4) *Cryptococcus guttulatus* Robin, now *Saccharomycopsis guttulatus* (Rob.) Schiönning (pag. 525, fig. 75).

There is no doubt that the Vuilleminian definition of the genus *Cryptococcus* in the medical sense, has been derivated from this chapter of Rivolta treatise (certainly well known to the french mycologist), and it must be antedated to the year 1873. Unfortunately, both quite clearly described species of *Cryptococcus* are referable, respectively, one to the genus *Histoplasma* Darling, while the other is the type species of the genus *Saccharomycopsis* Schiönning. But the same problem is offered by the type species of *Cryptococcus* emend. Vuillemin, namely *Cryptococcus hominis* Vuill., that in the opinion of many students is now attributed to the genus *Debaryomyces* as *D. neoformans* (Sanf.) Red., Cif. et Giord. (species that is not, of course, the type species of the genus *Debaryomyces*).

In conclusion, the Rivolta's amendment of *Cryptococcus* as a "medical" genus of asporigenous yeasts antedated the amendement of Vuillemin, while the definition of Robin is mixing together human and fermentative yeasts, only on the base of the cellular morphology. According Rivolta, *Cryptococcus* is considered distinct from *Saccharomyces* (*cerevisiae*) as not producing alcoholic fermentation, forming pellicle on liquid media, and found on diseased men or animals; but no valid type species is indicated, the better described species being, respectively, the type species of the genus *Saccharomycopsis* and one of the genus *Histoplasma*.

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[1]) *Crypta syphilitica* Salisbury, an "alga" identified with the "Syphilis corpuscles" of Lostoffer, is not considered as a true *Cryptococcus* (pag. 358—359 and fig. 61, 69 and 70), but it appears as "Cryptococcus" in the index.

Researches on the Etiological Agents of the American Blastomycosis.

II. Morphology and Systematic of the Agent of Posadas-Wernicke's Disease

(so called Coccidioidomycosis)

by

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(3.5.1951)

The principal aim of this paper is to explore the possibility of establishing a purely mycological diagnosis of the so called *Coccidioides immitis* (Stiles) Rixford and Gilchrist, 1896, without taking into consideration its origin, the aspects assumed by the fungus in its parasitic phase, or the reports of the clinical and anatomical lesions which it produces. In other words, we shall try to study and describe the morphology of the so called *C. immitis* in certain artificial culture media and determine whether such characteristics are sufficient by themselves to differentiate it from other fungi. This research seemed to us indispensable as most of the descriptions and illustrations so far known which have been obtained from cultures in artificial media fail to show any typical or pathognomonic characters; on the contrary, they represent non-specific aspects which can be met with in other fungi. There are of course exceptions, such as microphotograph 70 (p. 617) in Dubos' book (7) supplied to him by Conant; fig. 10 (microphotograph) of Nickerson (14) supplied by Emmons; fig. 104 (p. 165) of Swartz (17) supplied to him by Dubos (microphotograph); fig. 23 (p. 38) in Gohar's book (10) supplied to that author by Conant; the drawings of Negroni (13) and others which it is unnecessary to mention.

There is no doubt that the name *Coccidioides* originated not from an analysis of the aspect of the fungus in artificial culture media (in which its fructifications can be completely differentiated), but from its morphology in the parasitic phase. Indeed we believe, that this was the chief reason for the creation of a mono-generic and mono-specific family. The same thing has happened in the case of other fungi whose morphology in saprophytic phase is as yet imperfectly known. To cite one example among others that can be mentioned, we may refer to the parasite described by Fish (8) and by him classified (althrough provisionally) as *Ichthyosporidium hoferi* Plehn et Mulsow, 1911, despite the fact that in Fish's paper the mycological description is not sufficient for taxonomic purposes; the identification was made merely on the aspects assumed by the fungus in its parasitic phase and it is even impossible to state whether such aspects are pathognomic. Even imperfectly characterised, familia mono-genus and mono-species have been grouped into tribes.

As was emphasised in our first researches on the etiological agents of American Blastomycosis (2) we believe that besides the reasons here stated this taxonomic dislocation for the purpose of the medical diagnosis of certain pathogenic fungi was due also to the fact of the prevalent use of media rich in glucosides and proteins in which there is a predominant development of non-specific forms; the productions of constant and characteristic fructification being, in such conditions, frequently rare or anomalous.

It seems to us however that a diagnosis of the fungi and a classification based exclusively or mainly on the morphology they acquire in animal tissues can only be accepted provisionally while awaiting the knowledge of such morphological features in adequate artificial media as will allow of the organisation of a sound systematic classification. Considering the problem from a general taxonomic view-point and also from the point of view of the medical mycology there seems to us no reason for separating into different familia or genera fungi which, through presenting the same generic features in artificial culture media, are sometimes pathogenic and sometimes not; or show different morphological aspects in their parasitic phase in spite of the similarity of the clinical and anatomo-pathological pictures they give rise to. In other words, the pathogenecity and aspect off ungi in the parasitic phase without an analysis of their morphology in artificial culture media, do not seem to us a sufficient basis for creating new groupings or for serving as a basis for their mycological classification.

That, in certain cases, the use of appropriate media, especially poor culture media, may lead to the development of aspects and principally of fructifications which are constant and characteristic we are able to demonstrate in the paper cited above (2). In that paper the diagnosis of *Aleurisma brasiliensis* was possible because, in poor culture media, especially in EMMONS' media, appear constant elements of fructifications which either do not develop in rich culture media or only appear inconstantly. As we shall try to show in this paper, the causal agent of POSADAS-WERNICKE's disease also presents, constantly and characteristically, in EMMONS' medium, aspects which appear only irregularly and inconstantly in rich media such as SABOURAUD's or agar-blood. Such aspects, obtained in cornmeal agar, allow of a safe generic and specific diagnosis independently (we repeat) of the consideration of the morphology of the fungus in the tissues affected by the parasite or in the pathological products.

We may here state that the same characterisation is possible, in EMMONS' medium, in the case of the so-called *Blastomyces dermatitidis* which will be described in a subsequent paper.

Material used

In our investigations we have used the followings strains gratefully received from Colleagues, and also retrocultures obtained by inoculations in rats and guinea-pigs.

1) Culture n° 63 from the collection of L. Bogliolo and n° 22 from the collection of J. Aroeira Neves. Supplied by Dr. Amadeu da Silva Fialho of the Public Health Service of the Federal District, Rio de Janeiro. Origin unknown.
2) Culture n° 71 from the collection of L. Bogliolo and n° 23 from the collection of J. Aroeira Neves. Supplied by Prof. Oscar Versiani who received it from Dr. Ch. E. Smith of the Department of Public Health and Preventive Medicine, Stanford University School of Medicine, 2330 Clay Street, San Francisco, California, U.S.A. The strain was labelled P. W. and about it Dr. Smith wrote: "P. W. is quite virulent, 10—100 organisms being sufficient to kill a mouse". Sent on 28/10/1946 and received early in December, 1946.

3) Culture n° 72 from the collection of L. Bogliolo. Supplied by Prof. Oscar Versiani who received it from Dr. Ch. E. Smith. This strain was labelled "46" and about it Dr. Smith wrote: "has quite low virulence, mice sur, viving several hundred thousand". Sent on 28/10/1946 and received early in December, 1946.
4) Culture n° 79 from the collection of L. Bogliolo. Sent by Dr. Norman F. Conant, School of Medicine, Department of Bacteriology, Duke University, Durham, North Carolina, U.S.A. This strain was n° 972 of Dr. N. F. Conant's collection.
5) Culture n° 80 from the collection of L. Bogliolo and n° 23A from the collection of J. Aroeira Neves. Sent by Dr. Norman F. Conant. On the label was n° 974. Isolated from sputum. Sent in January 1947.
6) Culture n° 83 from the collection of L. Bogliolo and n° 23B from the collection of J. Aroeira Neves. Sent by Dr. Norman F. Conant through Dr. Paulo Borges. On the label n° 1009. Isolated from sputum. Sent in January, 1947.
7) Culture n° 213 from the collection of L. Bogliolo and 23C from the collection of J. Aroeira Neves. Sent by Dr. Norman F. Conant and received 21/11/1949. Labelled n° 2155. Isolated from sputum.
8) Culture n° 215 from the collection of L. Bogliolo and 23D from the collection of J. Aroeira Neves. Sent by Dr. Norman F. Conant and received 21/11/1949. On the label: n° 2150. Isolated from sputum.
9) Culture n° 216 from the collection of L. Bogliolo and n° 23E from the collection of J. Aroeira Neves. Received from Dr. Norman F. Conant on 21/11/1949. Labelled n° 2132. Systemic form.
10) Culture n° 219 from the collection of L. Bogliolo and 23F from the collection of J. Aroeira Neves. Sent by Dr. Norman F. Conant and received on 21/11/1949. Labelled n° 2158. Isolated from the fluid of the *cisterna magna*.
11) Culture n° 250 from the collection of L. Bogliolo and n° 23G from the collection of J. Aroeira Neves. Supplied by Prof. Oscar Versiani who received it from Dr. C. W. Emmons; isolated in 1941. The culture left Washington on 4/1/1950 and arrived at Belo Horizonte on 28/1/1950. On the label: n° 6210.

Culture n° 63 was pleomorphous and non-pathogenic; characteristic fructifications could not be obtained. Culture n° 72 was lost during the research. The other cultures are in the collection of the Division of the Institute of Hygiene and in the Department of Pathological Anatomy of the Faculty of Medicine of the U.M.G.

Characterisation, in artificial media, of the causal agent of Posadas-Wernicke's disease (called coccidioidomycosis; coccidioidal granuloma; valley fever; San Joaquim fever; desert rheumatism; etc.).

We used here the same technic employed for the study of *Aleurisma brasiliensis* (2) namely depositing the material (transfers and material obtained by inoculation in animals) not only in the usual manner but also taking care to place it on the spot were the medium adjoins the wall on the tube in its upper part. Direct observation through the culture tube with low or medium magnification. For greater details see the work on *A. brasiliensis* already mentioned (2). Besides EMMONS' cornmeal agar other culture media, were used chiefly Sabouraud with glucose or dextrose and agar-potato-dextrose (Bacto). The characteristic fructification was always more abundant and typical in cornmeal agar than in the other media so that, so far as we can see at present, EMMONS' medium represents the optimum for the fungus we are studying.

Other differences in the behaviour of the various media will be mentioned hereafter. For certain observations, preparations dissociated in lactophenol were made. For our observations no account was taken of the aspects assumed by the fungus in hanging drop cultures, since by this process the fungus develops in a closed medium and so the interchanges with the surrounding medium cannot take place as they normally should; on the contrary such interchanges take place with great facility in test tubes. Therefore in hanging drop cultures there must be a certain constraint of the fungus.

On the 20th or 30th days there begins to appear in the cultures the process of protoplasmic condensation in the hyphae. However, it is only after 50 or 60 days as a rule that the fructifications are abundant and well differentiated thus allowing of a safe diagnosis of the species. Of course there are slight differences between one strain and another.

1) *Macroscopic characters of the causal agent of Posadas-Wernicke's disease in cornmeal agar.*

Generally the fungus assumes macroscopically in cornmeal agar four principal aspects. However, strains which in one preparation present a certain type may in others show modifications and even in the same tube different macroscopic aspects may be met with (Fig. 1, d). Therefore it seems to us that it is for didactic purposes and in an effort to arrange them schematically that a description of them seems useful. We must however point out that the differences between one macroscopic type of *A. immitis* and another are less marked than in the various types of *A. brasiliensis* in cornmeal agar.

The four principal types observed by us were as follows:

a) In this group we include strains in which initially growth does not penetrate deeply and the stalk consists of prostrate creeping hyphae. In this type the aspect is pseudo-glabrous and satiny; and in cultures about 30 days old the borders are well defined, spread scantily along the wall of the tube. Later the formation of aerial hyphae is more abundant and they cover homogeneously the whole surface of the culture medium which then seems slightly cottony (Fig. 1, a). The colour is uniformly white. This aspect was not observed by us either in *A. brasilensis* or in *A. dermatitidis.*

b) In certain strains we observed the growth of prostrate hyphae around the point of the transfer and, at a certain distance from this point, an abundant development of markedly elevated, cotanilious compact white aerial hyphae. At times the whole appears irregularly circinate (Fig. 1, b). Later, the colonies become somewaht granular and pulverulent presenting an aspect intermediary between this type and that described in the following paragraph. In the cotanilious regions the fungus extends more abundantly along the wall of the tube. Some days, later, secondary colonies are formed in the lower part of the substratum, probably originating from the separation of fertile bits of the primitive culture. In the cultures observed by us these secondary colonies are more granular than the primary ones. Fig. 1, c, represents the same culture as Fig. 1, b, photographed 15 days later.

c) In this type the mycelium is less elevated than in the preceding one and the culture takes on a somewhat granular aspect (Fig. 1, a). Its colour is white. This granular type was observed by us in certain strains of *A. brasiliensis* and *A. dermatitidis* which however are pigmented. In the former fungus the surface of the culture approximates ivory yellow in colour (RIDGWAY, Pl. XXX, 21″. O—YY. f.); the posterior surface of the cultures approximates in colour to the following: auburn (RIDGWAY, Pl. II, 11. Orange. m.); chestnut brown (RIDGWAY, Pl. XIV, 11, Orange. m.); cinnamon brown (RIDGWAY, Pl. XV, 15′. Y—O. k.). In the latter fungus the surface of the culture is slightly yellowish and it is difficult to compare it to any of the standard colours described by RIDGWAY (15) but it approximates to light buff (Pl. XV, 17′. O—Y. f.). Looked at from the back the approximation is more definite. The same granular aspect but finer was seen by us in cornmeal agar in the case of *Trichophyton mentagrophytes*, in *Microsporum felineum* and in *Epidermophyton floccosum*.

d) In this type the mycelium largely takes on subsurface growth and it is only at a considerable distance from the point of the transfer that the hyphae become aerial but short. The aspect is glabrous. Where the hyphae become aerial they spread very little along the surface of the tube and the surface is somewhat lanuginous. For this reason we have been unable to represent it by means of photographs.

e) In certains cultures the above mentioned aspects are combined (Fig. 1, e).

f) All these types were studied by us in transfers received from the Colleagues mentioned when we referred to the material used for study. These transfers gave rise to colonies in which the above-mentioned aspects appear earlier than in the first cultures or transfers of *A. brasiliensis*. As has been said, its morphology was already perfectly characterised 20 or 30 days after the transfer.

g) Cultures made from pathological products of animals (guinea pigs and mice) inoculated with the above strains showed the aspects described above.

h) The cultures seen by us in SABOURAUD's medium and in potato-agar dextrose showed an always uniform aspect as described by most authors, it being impossible to distinguish different macroscopic types as happens on the contrary in the case of cornmeal agar. Growth was more rapid and the formation of aerial hyphae more abundant. In short, a uniformely cotanilious aspect.

2) *Microscopic characters in cornmeal agar.*

a) The characteristic and indeed the predominant fructification is represented by aleurias or aleurospores which are usually central (Pl. 2; Pl. 3, n° 1, 2, 3, 5, 6, 7, 9, 11, 13, 14, 15, 17, 18; Pl. 4, n° 1, 2, 3, 4, 5, 6, 15, 16; Pl. 5. n° 1, 7, 12, 13; and Figs. 2, 3, 4, 5, 6, 10; Pl. 6, n° 1 to 6, 10 to 13 and 16 to 20), less frequently lateral (Pl. 2; Pl. 3, n° 7, 13, 15, 17, 18; Pl. 4, n° 1, 3, 4, 6, 13, 15; Pl. 5, n° 2, 3, 8, 10, 12; Figs. 4, 5, 6, 10 and 11). The central forms appear in the shape of a small cylinder which seen from the front gives the impression of a rather regular rectangle (Pl. 3, n° 3 and 14; Pl. 6, n° 11, 12, 13; Fig. 2), sometimes one or two surfaces are concave or convex (Pl. 3, n° 1 and 7; Pl. 6, n° 1 and 2; Fig. 2); at other times the sides are equal and therefore the shape is more or less regularly quadrangular (fig. 2). More rarely it is ellipsoidal or spherical (Pl. 2; Pl. 3, n° 11 and 14; Pl. 5, n° 12; Fig. 5; Fig. 8). These aleurias may be seriated and separated from one another by empty spaces in the hypha which at times are fairly regular and short (Pl. 2; Pl. 3, n° 1, 3, 5, 14; Pl. 4, n° 1, 2, 5, 6; Pl. 6, n° 10 to 13; Figs. 2 and 6) or they may be longer and irregular in size (Pl. 2; Pl. 3, n° 2 and 11; Pl. 4, n° 13, 15, 16; Pl. 6, n° 2, 4, 6; Figs. 3, 4, 7). In the former case they are seriate and *grosso modo* resemble the beads of a rosary (Pl. 2; Pl. 3, n° 14; Pl. 6, n° 13, 18) and such are more frequent in relatively young cultures though at times met with in old ones, up to 30 months old. They are derived from segmentation of protoplasmic condensations (Pl. 1 and Pl. 2) which occupy more or less extensive sections of the hypha. In old cultures the sterile hyphae and also the fructifying hyphae become segmented and interlace and may form mixed irregular groupings as is shown in Plate 7.

However, the segmentation of these fragments does not always progress to a differentiation of the typical aleurias; indeed in fairly old cultures non-differentiated segments can still be observed (Pl. 7). In old cultures also, on account of the great dehydratation of the sterile parts of the hypha and the aleurias, these latter become more rounded and get closer together, the whole taking on the aspect found in *Scopulariopsis* (Pl. 7). When arranged laterally the aleurias sometimes are continous with a sterile segment of hypha (Pl. 2; Pl. 3, n° 11 and 17; Pl. 4, n° 1; Pl. 5, n° 8; Figs. 4, 6) or at times originate from a central aleuria (Pl. 2; Pl. 3, n° 7, 13, 15, 18; Pl. 4, n° 4, 6, 13, 15; Pl. 5, n° 8; Figs. 5, 6, 10, 11) or they originate from a section of fertile hypha (Pl. 3, n° 12, 13; Pl. 5, n° 8; Figs. 4, 5, 10). They are rarely pediculate and in this respect are sharply differentiated from those found in *Aleurisma dermatitidis* and *A. brasiliensis*. In the former the pedicles are as a rule longer and the aleurias more spheroidal, ellipsoidal or pyriform, while in *A. brasiliensis* they are more irregular, are preponderantly lateral but the pedicle is shorter than in *A. dermatitidis*. In the fungus now being studied the aleurias are at times arranged in the form of thyrsus (Pl. 2; Pl. 4, n° 3; Figs. 5, 6) or of bunch (Pl. 2; Pl. 4, n° 1, 7, 10, 13; fig. 2) as is the case, also, but less frequently with certain strains of *A. dermatitidis*. This arrangement is comparable with or analogous to that met with in certain fungi of the genus *Microsporum* and *Trichophyton*.

The dimensions of the aleurias of the so-called *Coccidioides immitis* are greater than those of the fungi belonging to the above mentioned genera or of the species *A. dermatitidis* and *A. brasiliensis*. However, there exist also very small aleurias but they form a small minority. This is due to the fact that the diameter of the mycelium of fructification is not constant; it is sometimes very small (Pl. 6, n° 18, 20).

b) The vegetative hyphae, which are irregularly septate, give rise to ramifications arranged laterally, frequently almost at right angles and these in their turn branch. It is from these ramifications that the apparatus of fructification or aleurophora arises by preference (Pl. 2; Pl. 4, n° 1, 2; Figs. 4, 5, 6, 7, 8); however, cytoplasmic condensations may form in the principal hyphae and in these the aleurias are differentiated (Pl. 2; Figs. 4, 5, 6, 8). Not rarely there are seen anastomoses or confrontations between vegetative hyphae or between sterile hyphae or between both types that is between vegetative and vegetative and between sterile and sterile and reciprocally (Pl. 2; Pl. 4, n° 1, 2, 4, 5; Pl. 5, n° 8; Figs. 4, 5, 7, 8).

In these vegetative hyphae, in certain strains, there exist racquet formations (Pl. 3, n° 16), chlamydospores (Pl. 3, n° 4), terminal mycelial dilatations (Pl. 4, n° 10, 11, 12, 13; Figs. 18, 19); such formations have already been noted by most authors. There are also found mycelial candelabra-like terminations (Pl. 5, n° 11, 13) and sometimes mycelial ramifications which remind one of the pectinate organs of *Achorion* (Pl. 5, n° 5, 6). The vegetative hyphae are of different thicknesses some measure 3—4 μ while beside them are found others having a half or one third this diameter. Sometimes the same filament changes its diameter several times; in other cases a relatively thick hypha gives rise to a thin branch or vice-versa.

c) In certain strains spiral formations exist in abundance (Pl. 4, n° 19, 20, 21, 22; Pl. 5, n° 3; Figs. 2, 9, 14, 15, 16, 17) comparable to those found in certain *Trichophyton gipseum*, microsporons and epidermofitons, but not observed by us, up to now, in *A. brasiliensis* and *A. dermatitidis*.

There exist also hyphae in the form of long or short loose spirals as in *A. dermatitidis* and *A. brasiliensis*.

d) There are met with also between two branches of a principal vegetative hypha or between two principal vegetative hyphae, multiple anastomoses forming a kind of grouping skein or labyrinth (Pl. 5, n° 2, 4) consisting of bent angular sterile or fructified hyphae resembling at times the aspects registered by SABOURAUD (16) in Fig. 307, U, of *Trichophyton radiolatum*. We believe these formations to be artefacts produced by the evaporation of drops of the exeresis water in consequence of which the stalk developing outside the culture medium on the side of the tube, suffers rapid desiccation.

e) All these differentiated forms of the fungus under consideration are met with not only in cornmeal agar but also in culture media rich in carbohydrates and proteins; but, as we have said, in such media, the formation of sterile vegetative mycelium is more abundant and there are more racquet formations and chlamydospores than typical fructifications, the aleurias. In this respect *A. immitis* differs from *A. brasiliensis* which in richa media, very rarely shows the typical fructifications, aleurias.

f) In the prostrate and aerial hyphae, in our strains, we have never been able to observe fructifications which could be interpreted as asci or any form more differentiated than aleurias. Similarly, such fructifications were not observed in *Aleurisma dermatitidis* and *A. brasiliensis*.

Discussion

Our aim in these researches is not only to study the morphology of the causal agent of POSADAS-WERNICKE's disease, but also to determine the possibility of establishing the systematic position of the fungus. In this connection it seems obvious to us that its taxonomy cannot depend, at least for the time being, on its aspects in the parasitic phase which indeed are imperfectly known. On the contrary, it should be based predominantly, or at least in equal proportion, on a knowledge of its saprophytic phase. It is indisputable that the consideration of the aspects of the fungus in its parasitic phase have influenced to a perhaps undue degree, those who have studied the subject. In our opinion it is therefore indispensable to analyse the type of spore which predominates over the other organs of propagation of the fungus so as to allow us to characterise it. In other words it is necessary to determine whether the spore is an aleuria or aleurospore, an arthrospore, a true conidia or an endospore.

As a rule the descriptions given by most authors of the microscopical morphology of the fungus in cultures are confused and cannot serve as a guide to diagnosis. In general authors speak of the production of arthrospores, of pseudo-arthrospores, of endospores, of conidia (or arthrosonidia) and some speak of the formation of asci.

CONANT, STOVER, MARTIN, BAKER and CALLAWAY (4) as well as the majority of authors speak of the formation of arthrospores although their descriptions are generally very summary. DODGE's description (6), for example, seems to us insufficient for taxonomic purposes for he refers to the fungus under consideration in the following terms: "hyphae which can give rise to racquet-shaped, mycelia, chlamydospores, cells giving rise to arthospores and then refers to asci measuring 4 to 80 μ. MOORE also speaks of asci (12) and DODGE in his book gives drawings of forms which can be interpreted as such (l.c.,

p. 150, fig. 24, n° 19). However it is not impossible, in our opinion, that the forms seen and drawn by MOORE are nothing but spherical cells, resistant forms (chlamydospores) full of fatty drops such as are met with in culture media rich in proteins and polysaccharides, as for example LOEFFLER's medium, agar-blood, etc.. Such forms are more commonly met with in those stems which in the circumstances above mentioned, or in conditions of suffering, take on the type *Achorion*; they are glabrous and easily detached from the substratum. In the above mentioned media we also have seen the cells interpreted by MOORE as asci, but they were completely full of lipoids which stained by election with Sudan III and Scarlet R. Hence we have interpreted them as resistant forms (chlamydospores). We have also met with them in *Aleurisma dermatitidis* as will be described by us in a future paper. It is convenient to state here that in our material we have never met with aspects which could positively be interpreted as copulative forms resulting in the formation of ascogonias or elements of a similar appearance, such as would approximate the causal agent of POSADAS-WERNICKE's Disease (so called *Coccidioides immitis*) either to the *Spermophthoraceae* or to the higher *Ascomycetes*. In our opinion no positive proof of copulation has been forthcoming. The illustrations published by REDAELLI and CIFERRI (3) may equally well be interpreted as copulation or as direct division, by gemmation for example.

Among other authors, AGOSTINI (1) mentions true conidia and fructifications which he regards as arthroconidia, but his description is absolutely insufficient for a mycological diagnosis. NEGRONI (13) believes that the characteristic fructification of the causal agent of POSADAS-WERNICKE's disease is an endospore. Some authors, influenced possibly by the forms the fungus assumes in its parasitic phase, base their classification chiefly on this phase. Thus LANGERON (11) compares the so-called *C. immitis* with *Ichthyophonus hoferi* which has been studied by DANIEL (5) and which is a fungus met with in the herring (*Clupea harengus*); and this in spite of the fact that this fish parasite differs widely in its parasitic life from the so-called *C. immitis* and in spite of our practically complete ignorance of its saprophytic life.

If our descriptions and even our illustrations are comparable with those of some other authors, our interpretation differs from that of others as regards the significance of the spores and consequently of the term which should be used to indicate them and also as regards the systematic position of the fungus. The object of the following therefore is to inform our readers as regards the exact terminology used by us in our studies.

The aleurias or aleurospores which were precisely defined by VUILLEMIN (18, 19) are similar to conidia or conidiospores in their functions, position and uniformity. But whereas the conidia, though originating from the thallus are essentially caducous, the aleurias are thallospores which become liberated only by the death of the hypha or thallus in which they become differentiated by the phenomenon of cytoplasmic condensation. The affinity of aleurias to the chlamydospores is shown by their continuity with the thallus, by their non-dehiscence and by the intermediate forms which by insensible gradations join them to intercalary and polymorphous chlamydospores. Hence, as they are not chlamydospores — nor yet conidias, they get their special designation — aleuria.

The differentiation of the aleurias from the arthrospores (improperly called oidia) is made by the fact that in the latter dissemination is automatic, since arthrospores are liberated in consequence of jellification and autolyses of the median layer or septa separating the arthros; while, in aleuria dissemination is passive. Another point of distinction is that the formation of arthrospores is not preceded by the phenomenon of cytoplasmic condensation as is the case with aleurospores. Aleurospores differs from conidia and arthrospores in that they always show in their extremities vestiges or remains of the hypha which died and liberated the aleurias. Such vestiges are not observed in conidia and arthrospores.

Endospores, in their turn, according to LANGERON (11), should be thallospores or spores contained within the thallus which are liberated either by desarticulation or by splitting of the hypha wall which in one way or another must break down in order to liberate the endospores. As in the case of the arthrospores, dissemination of endospores is active. Apart from the fact that the endospores are as yet little known or studied (according the LANGERON certain formations which at the same time called endoconidias cannot be considered as endospores) there remains the circumstance that the aleurias, like the chlamidospores, are thallospores whose dissemination is passive and late and which are produced by the process of cytoplasmic condensation which is not the case with the so-called endospores. Therefore, even if we wish to include among the imperfect spores the so-called endospores, which are not met with in VUILLEMIN's original classification, the fact still remains that these thallospores, for so they should be considered according to LANGERON, have an origin, mode of formation and morphology different from the aleurias or aleurospores. In our observations directly in the culture tube (and therefore in the best conditions for study) and in preparations stained or unstained made by dissociation in lacto-phenol, we were able to follow the constant formation of fructifications which became differentiated through the mechanism of cytoplasmic condensation in the causal agent of LUTZ' disease and in GILCHRIST's disease. The fructifications of the causal agent of these three diseases are essentially non-caducous, are disseminated passively and remain adherent to the mycelium even when the latter is dead and dehydrated forming in old cultures a kind of floury deposit on the surface of the colonies. When the fructifications are separated from the mycelium, either artificially by dissociation and dissection or naturally by rupture of a dead empty section of the stem, there are always met with at their extremities, remains or residues of the empty part of the hypha tube as shown in our illustrations and even in those of NEGRONI (see especially figs. 1, 2, by that author); NEGRONI however interprets these thallospores as endospores. They are therefore aleurias.

It is our opinion that the aleurias of the causal agent of POSADAS-WERNICKE's disease (and also that of LUTZ' disease and GILCHRIST's disease) are formed in the same manner as in the case of the fungus of the genus *Trichophyton* as well as of *Microsporum* and *Achorion*. We shall also demonstrate that a certain degree of relationship exists between the fungi of the American hypho-blastomycoses, to employ LUTZ' happy nomenclature (11, a) and various agents of tinea as can be seen from as examination of the table below (Tab. 1) which shows a comparative list of the various differentiated organs common to the so-called American blastomycosis and tinea.

It is convenient to repeat here that in the causal agent of POSADAS-WERNICKE's disease arthrospores were never observed by us, not at least as they are met with in the genera *Geotrichum*, *Trichosporum* and *Oidiodendrum*.

As will be seen from the above, in the characterisation of imperfect spores and in considering their utility for classification and identification of the fungus, we have adopted the ideas of VUILLEMIN (18, 19) which we consider practical and logical. Therefore we have, in accordance with VUILLEMIN, divided imperfect spores into two categories: *a*) non-caducous thallospores, formed at the expense of the thallus by transformation of preexisting elements; *b*) conidia or conidiospores, which are terminal, lateral or external spores which are essentially caducous, originate from the thallus and are consequently newly formed elements.

Five tipes of thallospores are distinguished, namely: aleurias, arthrospores, blastospores, dictiospores and chlamydospores. It is well know that blastospores originate by the sprouting of a pre-exisisting element forming subsequently a pseudo-mycelium; they are met with in fungi of the genus *Candida* and in leaven-like fungi. The dictiospores are of no interest here. We shall however define the chlamydospores as a fairly bulky dilatation of the thallus, with thick walls and containing lipoids and other substances of reserve. Relatively to the hypha from which it sprang, the chlamydospores may be intercalary, lateral or terminal; in the first case it is separated from the hypha by two septa, in the other two cases by only one. In spite of fact that chlamydospores are met with in nearly all the fungus familia, alone or associated with other spores, their presence is not sufficient for a mycological diagnosis.

Table 1

Names of species	Spores and other formations													
	Simple aleurias	Bi or pluri celular aleurias	Racquet shaped hyphae	True spirals	Loose spirals	Candelabra	Chlamydospores				Nodular organs	Mycelial arborescences	Thyrsis	Compound bunches
							Intercalary	Lateral	Terminal	Catenulate				
Aleurisma brasiliensis (*)	+	—	—	—	+	+	+	+	+	+	—	+	+	—
Aleurisma dermatitidis (**)	+	—	—	—	+	+	+	+	+	+	—	+	+	—
Aleurisma immitis	+	—	+	+	+	+	+	+	+	+	—	+	+	+
Trichophyton denticulatum	+	—	—	—	—	—	+	+	+	+	—	—	+	+
Trichophyton glabrum	+	—	—	—	—	—	+	+	+	+	—	—	+	—
Trichophyton fumatum	+	—	—	—	—	—	+	+	+	+	—	—	+	+
Trichophyton rubrum	+	—	—	—	+	—	+	+	+	+	—	—	+	+
Trichophyton mentagrophytes	+	+	—	+	—	—	+	+	+	+	—	—	+	+
Trichophyton cerebriforme	+	—	—	—	—	—	+	+	+	+	—	—	+	+
Trichophyton sulfureum	+	—	—	—	—	—	+	+	+	+	—	—	+	+
Trichophyton multicolor	+	+	—	—	+	—	+	+	+	+	—	—	+	+
Microsporum felineum	+	+	+	+	+	—	+	+	+	+	—	—	+	—
Microsporum fulvum	+	+	+	+	+	—	+	+	+	+	+	—	+	+
Achorion gallinae	+	+	+	—	+	+	+	+	+	+	—	—	+	+
Achorion Schoenleini	+	+	+	—	—	+	+	+	+	+	+	+	+	+

Observations

(*) The aleurias are at times arranged in groups giving the appearance of compound bunches.
(**) Branching and extremely simplified bunches.

NOTE — The aleurias of *A. brasiliensis* show in their fertile hyphae the same arrangement met with in *Achorion gallinae*. Sabouraud called them external spores (*l.c.* pg. 708, fig. 412).

In certain cultures of *A. dermatitidis* the fructification is very similar to that of *Achorion Quinckeanum* (Sabourad, *l.c.*, pg. 706—707, fig. 409 e 410).

Arthrospores have already been defined and it has been said that they are found among others in the genera *Geotricum, Trichosporum* and *Oidiodendrum*. We have also stated that we have never met with them in the causal agent of POSADAS-WERNICKE's disease.

Aleurias also, as is well known, are met with in many other fungi. But in the causal agent of POSADAS-WERNICKE's disease, in artificial culture media, and especially in media poor in proteins, glucides etc., the aleurias are not only absolutely predominant, but also possess characteristics different from the aleurias of *Aleurisma brasiliensis* and *Aleurisma dermatitidis* (2) as well as from the aleurias of the fungi belonging to the genera *Trichophiton, Microsporum* and *Achorion* as we shall demonstrate in another paper. For all these reasons and taking into consideration also the fact that the aspects of the parasitic phase (still imperfectly known) cannot serve as a basis for a mycological classification, we are of opinion that the causal agent of POSADAS-WERNICKE's disease should be classified as *Alerisma immitis*, if the genus *Aleurisma* is considered valid.

The diagnosis of the fungus under consideration is as follows:

Aleurisma immitis. — In substratis pauperibus gignuntur hyphis mycelicis hyalinis, septatis, satis ramosis, ramulis in angulo pene recto; aleuriis abundantissimis, valde simplicibus, centralibus et regulariter seriatis, vel apicalibus, rarissime lateralibus, sessilibus, rare pedicellatis, rectangularibus, hyalinis, aliquoties in racemis et thyrsis dispositis. Spirae saepe videntur. In substratis carbohydratis plenis, chlamydosporis intercalaribus et apicalibus abundantibus; aleuriis et spiris nec numerosis aut typicis quam in pauperibus substratis.

Summary and conclusion

We have been able to establish typical characteristics of the species, in artificial culture media, of the fungus causing POSADAS-WERNICKE's disease. Our work is base on the study of ten strains of the fungus and on their retrocultures — obtianed by inoculation of these strains in laboratory animals — in variosu culture media, among them cornmeal agar (EMMONS media) which in our opinion is a medium indispensable for the differencing and for the classification of the fungus from other agents which produce syndromes of a like nature.

A revision was made of the literature of the morphology of the causal agent of POSADAS-WERNICKE's disease and an analysis of the predominant and characteristic fructification of the fungus. In attention to the above facts, we think that the fungus in question should be included in the genus *Aleurisma* LINK, 1809. Therefore, admitting the validity of this genus, we believe that the name of the etiological agent of POSADAS-WERNICKE's disease should be changed to *Aleurisma immitis*, the diagnosis being justified by the microscopic study in EMMONS media and completed by the forms found in a medium rich in glycides. The diagnoses is the following:

Aleurisma immitis. — In substratis pauperibus gignuntur hyphis mycelicis hyalinis, septatis, satis ramosis, ramulis in angulo pene recto; aleuriis abundantissimis, valde simplicibus, centralibus et regulariter seriatis, vel apicalibus, rarissime lateralibus, sessilibus, rare pedicellatis, rectangularibus, hyalinis, aliquoties in racemis et thyrsis dispositis. Spirae saepe videntur. In substratis carbohydratis plenis, chlamydosporis intercalaribus et apicalibus abundantibus; aleuriis et spiris nec numerosis aut typicis quam in pauperibus substratis.

Synonymy

Megalocytosporides WERNICKE, R., 1892.
Ueber eine Protozoenbefund bei Mycosis fungoides? *Centralbl. f. Bakt.*, **12**: 859—861; Pl. 6. And: POSADAS, A., 1897. — Ueber eine neue durch Protozoen verursachte und auf Thiere uebertragbare am Menschen. Infectioese generalisierte Psorospermosis (Buenos Aires). *Montschr. f. Prakt. Dermat.*, **27**: 593. And: POSADAS, A., 1898. — Ensayo sobre una nueva neoplasia del hombre producida por un protozoario y transmisible a los animales. *Comunicacion a la Academia de Buenos Aires.* Jacobo Peuser, Buenos Aires. And: POSADAS, A., 1900. — Psorospermiose infectante généralisée; *Rev. Chirurg.*, **21**: 277—282: 7 figs.

Coccidioides immitis STILES, G. W. M. S. SH, in RIXFORD, E. et GILCHRIST, T., 1896.
Two cases of protozoan (coccidioidal) infection of the skin and other organs. I: First case: protozoan or coccidioidal pseudotuberculosis. II: A second case of protozoan infection. *John Hopkins Hosp. Reports*, **1**: 209—268: Pl. 21-43.

Coccidioides pyogenes STILES, G. W. M. S. SH. in RIXFORD, E. et GILCHRIST, T., 1896.
Two cases of protozoan (coccidioidal) infection of the skin and other organs. I: First case: protozoan or coccidioidal pseudo-tuberculosis. II: A second case of protozoan infection. *Johns Hopkins Hosp. Reports*, **1**: 209—268: Pl. 21—43.

Coccidioides neoplasicum CANTON, E., 1897.
Tratado de los zooparasitos del cuerpo humano, Buenos Ayres, p. 108.

Posadasia sphaeriformis ("esferiformis") CANTON, E., in POSADAS, A., 1898.
Psorospermiosis infectante generalizada. Primeiro Congresso Latino-Americano de Medicina. Buenos Aires.

NOTE. — The name "esferiformis" given by CANTON to the causal agent of Posadas-Wernicke's disease was emended by CIFERRI, R. and REDAELLI, P. to "spheriformis" (3). Besides these two authors justly consider "the genus of CANTON invalid because *nomen nudum* and because apparently it was published not in print by in mimeographed sheets".

Coccidioides immitis pyogenes OPHULS, W. et MOFFIT, H. C., 1900.
A new pathogenic mould (formerly described as a protozoan) (*Coccidioides immitis pyogenes*). Preliminary report, *Philadelphia Med. Journal*, **5**: 1471—1472.

? *Oidium protozoides* OPHÜLS, W., 1905.
Further observations on a pathogenic mould (further described as a protozoan, *Coccidioides immitis, Coccidioides pyogenes*). *J. Exper. Med.*, **6**: 443—485: Pl. 34—38.

Oidium coccidioides OPHÜLS, W., 1905.
Further observations on a pathogenic mould (formerly described as a protozoan, *Coccidioides immitis, Coccidioides pyogenes*). *J. Exper. Med.*, **6**: 443—485: Pl. 34—38.

Oidium immite VERDUN, 1907.

NOTE. — Quoted by FONSECA, O., Filho (see the bibliography). We were unable to read the original work.

Oidium pyogenes VERDUN, 1907.

NOTE. — Quoted by CIFERRI, R. and REDAELLI, P. (see bibliography). We were unable to read the original work.

Blastoporidium Schooi HARTMANN, M., 1912.
Demonstration eines neuer Menchen pathogen Protiste. *Zentr. f. Bakt., I Abt.*, Ref., **54**, Suppl., 253.

Zymonema immitis MELLO, F. De et FERNANDES, L. G., 1918.
Ensaio de classificação de fungos pertencentes à classe dos Blastomyces com descrição de um genero e algumas especies novas. *Arq. Hyg. Pat. Exot.*, **6**: 278—279 and 283—284.

Mycoderma immite VERDUN et MANDOUL, 1924.
Précis de Parasitologie, p. 769—771.

Mycoderma immite (RIXFORD et GILCHRIST) BRUMPT, E. et auct., 1927.
In: BRUMPT, E., Précis de Parasitologie, Masson e Cie., Paris, 1936, p. 2023. And in: BRUMPT, E., Précis de Parasitologie, Masson e Cie., Paris, 1949, p. 1990.

Blastomycoides immitis CASTELLANI, A., 1928.
Consideration on the fungi found in blastomycosis. *Am. Journ. Trop. Med.*, **8**: 381—388. And: *Am. Med.*, **23**: 290—291: 1928.

NOTE. — It seems to be the same fungus described as *Geotricum immite* by AGOSTINI, A., 1932, Observations on fungi found in cases of North American blastomycosis of the skin and lungs. *The Journ. of Trop. Med. a. Hyg.*, **35**: 266—269: 3 figs.; and in 1934: Su due ifomiceti isolati da micosi umane. "*Blastomycoides immitis*" (RIXFORD and GILCHRIST) CASTELLANI, A., e "*Blastomycoides tulanensis*" CASTELLANI, A., *Atti. Ist. Bot. R. Univ. di Pavia*, serie IV, **3**: 9—15: 3 figs. But it should be noted that DODGE, C. W., (l.c., p. 168) considers *Blastomycoides dermatitidis* as synonymous with *Blastomyces dermatitidis* (= *Zymonema dermatitidis*).

? *Blastomycoides dermatitidis* CASTELLANI, A., 1928.
Considerations on the fungi found in blastomycosis. *Am. Med.*, **34**: 289—295; and *Am. Journ. Trop. a. Med.*, **8**: 281—388.

NOTE. — DODGE, C. W. (l.c., p. 168) considers it as synonymous with *Blastomyces dermatitidis* (= Zymonema dermatitidis). On the contrary, CIFERRI, R. and REDAELLI, P. (l.c., p. 410) have studed and considered a strain of *Blastomyces dermatitidis* (GILCHRIST et STOKES), from the collection of CASTELLANI, A., as a legitimate example of *Aleurisma immitis* (= *Coccidioides immitis*).

Pseucoccidioides Mazzai FONSECA, O., Filho, 1928.
In: Parasitologia Medica, 1943, Vol. I, pp. 242—245.

? *Geotricum immite* AGOSTINI, A., 1932.
Observations on fungi found in cases of North American Blastomycosis of the Skin and Lungs. *The Journ. of Trop. Med. a. Hyg.* **35**: 266—269: 3 figs. And: Su due ifomiceti isolati da micosi umane: "*Blastomycoides immitis*" (RIXFORD et GILCHRIST) CASTELLANI, A., e "*Blastomycoides tulanensis*" CASTELLANI, A., *Atti Instituto Bot. di Pavia*, serie IV, **3**: 9—15: fig. 3.

NOTE. — CASTELLANI, A. (Observations on fungi isolated from cases of blastomycosis cutis and blastomycosis pulmonalis in North America and Europe. Remarks on Blastomycetin. *The Journ. of Trop. Med. a. Hyg.*, **36**: 297—321: 1933) publishes in this work figs. 4a—11 as characteristic of this fungus. Fig. 4a is the photograph of a culture tube (glucose agar) in which no specific or generic characteristics can be distinguished. Fig. 10 reproduces the macroscopic lesion and is equally noncharacteristic. Figs. 5, 6, 7, 8 are microphotographs; like figs. 9 and 11 which are drawings, they do not admit of any mycological diagnosis whatever.

Coccidioides esferiformis Moore, M., 1932.
Coccidioidal granuloma: a classification of the causative agent, *Coccidioides immitis. Ann. Missouri Bot. Gard.*, **19**: 397—428: Pl. 25.

NOTE. — See what has been written about *Posadasia sphaeriformis* (*"esferiformis"*) Canton, E., 1898.

? *Monosporium tulanense* (Castellani, A., 1926) in Agostini, A., 1932.
Observations on fungi found in North American Blastomycosis of skin and lungs. *Journ. of Trop. Med. a. Hyg.*, **32**: 266—269: 3 figs. And: Su due ifomiceti isolati da micosi umane: *"Blastomycoides immitis"* (Rixford et Gilchrist) Castellani, A., e *"Blastomycoides tulanensis"* Castellani, A., Atti Ist. Botanico R. Univ. di Pavia, Serie IV, **4**: 9—15: 3 figs.

NOTE. — Castellani, A. Observations on fungi isolated from cases of blastomycosis cutis and blastomycosis pulmonalis in North America and Europe. Remarks on Blastomycetin. *Journ. of Trop. Med. a. Hyg.*, **36**: 297—321: 1933: 56 figs., Pl. 1. The author dedicates figs. 17, 17a, 18, 19, 19a, 20, 21 and 22 to the illustration of this fungus. Fig. 17 represents the macroscopic aspect in culture media (not specified). Fig. 21 shows the cutaneous ulcerations. Figs. 17a, 18 and 19 are microphotographs and 19a, 20 and 22 are drawings. The photographs and drawings seem to show that we are dealing with *Blastomyces dermatitidis* (= *Aleurisma dermatitidis*) to which we shall devote a subsequent work (Researches on the Etiological agents of the American Blastomycosis. III. Morphology and Systematic of Gilchrist's Disease).

It should be noted that Dodge considers the organism called *Monosporium tulanense* as a synonymous of the fungus denominated *Aleurisma tulanense* Ota, M. et Kawatsure, S., 1933 (*Arch. Derm. Syphilis*, **169**: 156—160).

? *Geotrichum dermatitidis* Castellani, A., 1933.
Observations on fungi isolated from cases of blastomycosis cutis and blastomycosis pulmonalis in North America and Europe. Remarks on Blastomycetin. *J. Trop. Med. a. Hyg.*, **36**: 297—321: figs. 56: Pl. 1.

NOTE. — According to Ciferri, R., and Redaelli, P. (l.c., pg. 459), this fungus should not be confused with the causal agent of Gilchrist's disease that is *A. dermatitidis* (= *Blastomyces dermatitidis*).

? *Geotrichum louisianoideum* Castellani, A., 1933.
Observations on fungi isolated from cases of blastomycosis cutis and blastomycosis pulmonalis in North America and Europe. Remarks on Blastomycetin. *J. Trop. Med. a. Hyg.*, **36**: 297—321: fig. 56: Pl. 1.

NOTE. — Considered by Ciferri, R. and Redaelli, P., (l.c., p. 410) to possess a *facies* indistinguishable from strains which undoubtedly are *Coccidioides immitis* Stiles in Rixford et Gilchrist, 1896.

However, according to Castellani himself (l.c., pg. 304): "this fungus differs from *G. immite* and *G. dermatitidis* in the mycelial filaments being very wavy and in the enormous number of very short arthrospores present in old cultures". To illustrate and demonstrate the so-called *G. louisianoideum*, Castellani, A., dedicates fig. 16 (l.c., pg. 304) which however in our opinion is insufficient for a mycological diagnosis.

? *Glenospora meteuropea* Castellani, A., 1933.
Blastomycosis: a short general account (contains description of seven new species of fungi producing the disease). *Med. Press and Circular*, **136**: 440; And: Observation on fungi isolated from cases of blastomycosis pulmonalis on North America and Europe. Remarks on Blastomycetin. *Journ. Trop. Med. and Hyg.*, **36**: 297—321: 56 figs.: Pl. 1.

NOTE. — CIFERRI, R. and REDAELLI, P. (l.c., pg. 410) studied two strains of this fungus; one isolated by CASTELLANI in Southern Italy, the other in the Balkans. The latter, which was isolated in the Balkans, according to the above-mentioned authors, "did not prove to be *Coccidioidis immitis*". Besides DODGE (l.c., p. 790) considers that the fungus called *Glenospora meteuropea* CASTELLANI, 1933 is doubtful in its systematic position and calls it *Aleurisma meteuropeum* (CASTELLANI) DODGE, *n. comb.*

In our opinion figs. 30 to 33 in CASTELLANI's work are insufficient for any mycological diagnosis.

? *Geotrichum immitis* (GILCHRIST and STOKES, 1898) CASTELLANI, A., 1933. Observations on fungi isolated from cases of Blastomycosis cutis and Blastomycosis pulmonalis in North America and Europe. Remarks on Blastomycetin. *The Journ. of Trop. Med. and Hyg.*, **34**: 297—321: 56 figs.: Pl. 1.

NOTE. — The author in this work dedicates to this fungus figs. 11a to 15. The first is the macroscopic aspect in culture medium (glucose-agar) but does not show any characteristic which can distinguish it from the so-called *Geotrichum immite* AGOSTINI, A., 1932, for example. Figs. 12, 13 and 14 are microphographs and, like fig. 15 (drawing), show aspects which are absolutely non-specific. No fructifications, not even of the most primitive kind, are seen; they are therefore insufficient for any mycological diagnosis. CIFERRI, R. and REDAELLI, P. (l.c., p. 409) also say that the identification of *Coccidioides immitis* with *Geotrichum*, made by AGOSTINI, must be attributed to the fact that the author worked only with cultures *in vitro* and therefore found herself "unable to observe the endosporulant forms of the fungus".

? *Coccidioides immitis var. metaeuropeus* (CASTELLANI, A.) CIFFERRI, R. et REDAELLI, P., 1934.
Quoted by CIFERRI, R., (l.c., 1936, p. 459).

NOTE. — CIFERRI, R. and REDAELLI, P. (l.c., pg. 459) makes the organism synonymous with *Glenospora metaeuropea* CASTELLANI, A., 1933 (according to the quotation of CIFERRI and REDAELLI).

Coccidioides immitis typicus CIFFERRI, R., et REDAELLI, P., 1934.
In: CIFERRI, R. and REDAELLI, P., 1936, l.c., pg. 459.

Coccidioides immitis Pipkini CIFFERRI, R., et REDAELLI, P., 1934.
In: CIFERRI, R., and REDAELLI, P., 1936, l.c., pg. 459.

? *Coccidioides immitis metaeuropeus* (CASTELLANI, A.) CIFERRI, R., et REDAELLI, P., 1934.
In: CIFERRI, R., and REDAELLI, P., 1936, l.c., pg. 459.

NOTE. — CIFERRI and REDAELLI (l.c., p. 459) consider this organism as synonymous with *Glenospora meteuropea* CASTELLANI, A., 1933, of which mention was made above.

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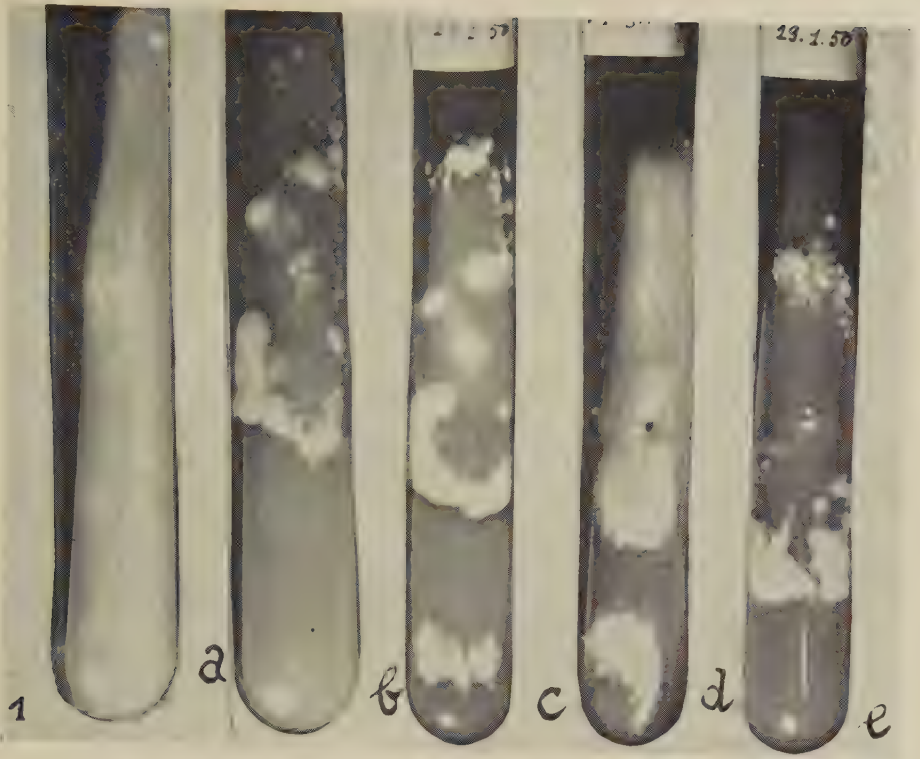
Explanation of the figures

Fig. 1. Macroscopic aspects of the causing agent of Posada-Wernicke's disease on cornmeal-agar: *a*) Strain n° 8, 24 days old. Thallus preferently prostrate, rarely aerial, more easily seen in oblique plane and at the ends of the surface of the culture media; *b*) Strain n° 80, 24 days old. Culture partly prostrate and with abundant formation of aerial hyphae, giving this last zone a cottonlike aspect; *c*) Strain n° 9, 24 days old. The same culture of fig. 1*b*, photographed 15 days later, to show the secondary cultures, probably derived from fertile portions detached from the primitive culture. The secondary culture show an aspect rather granulous (see fig. 1, *d*); *d*) Strains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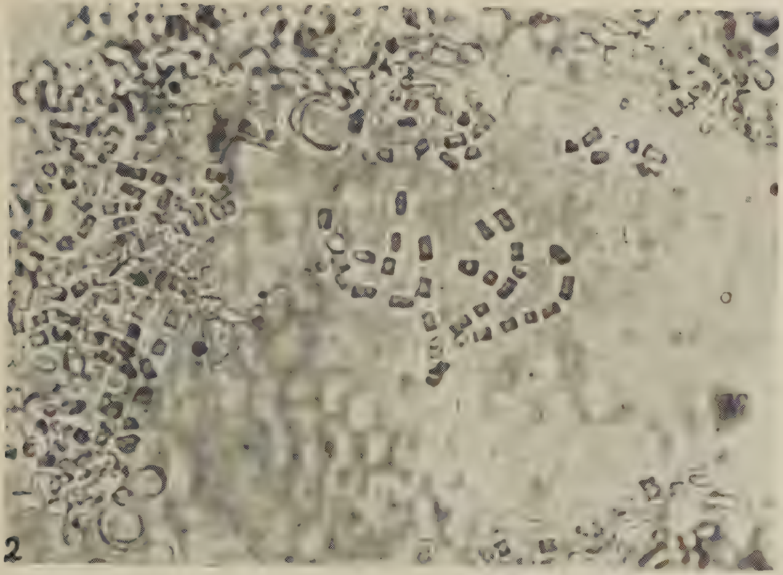
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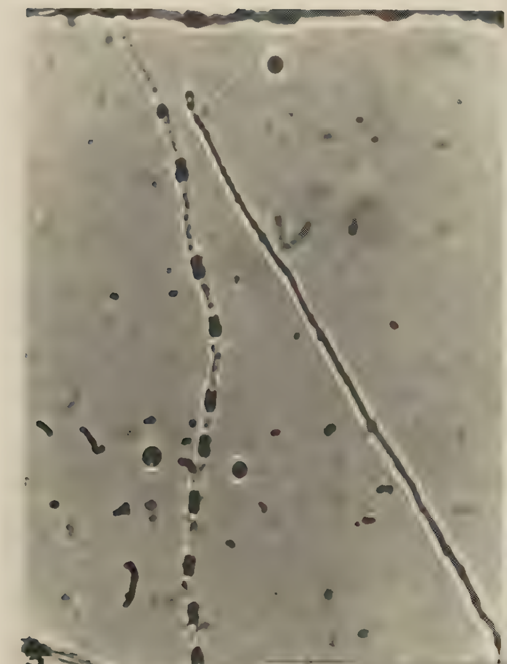
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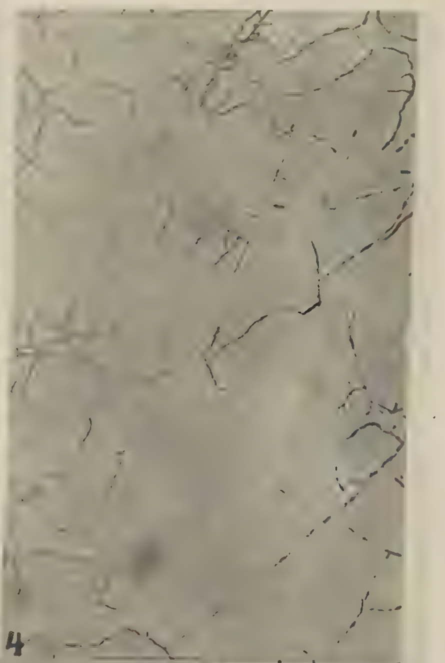
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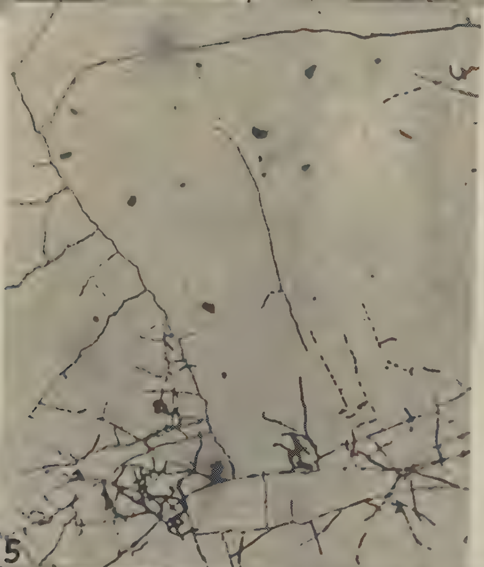
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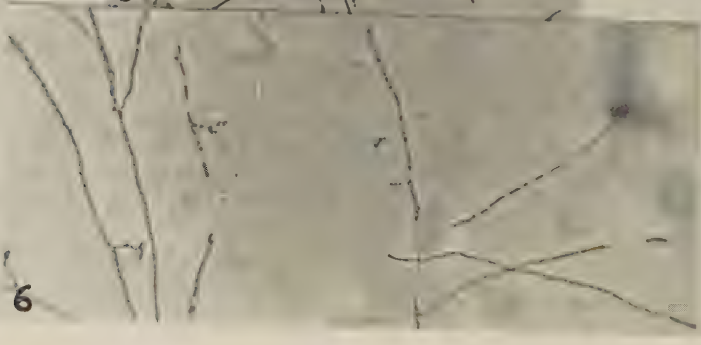
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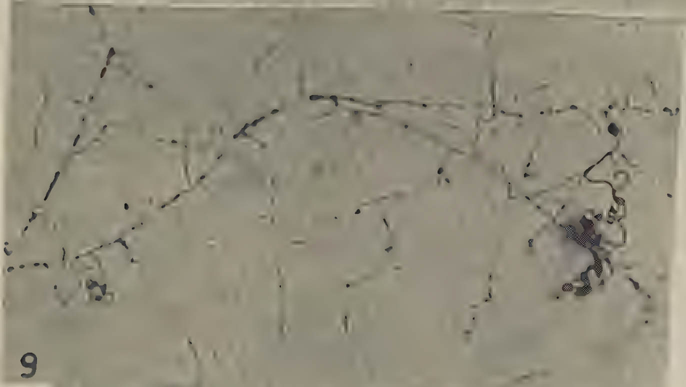

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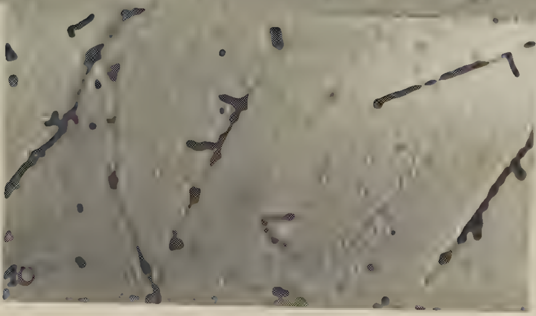

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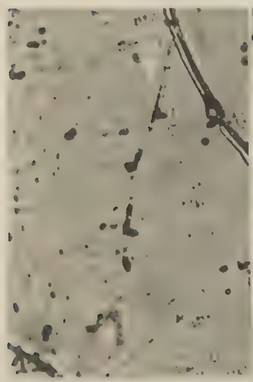

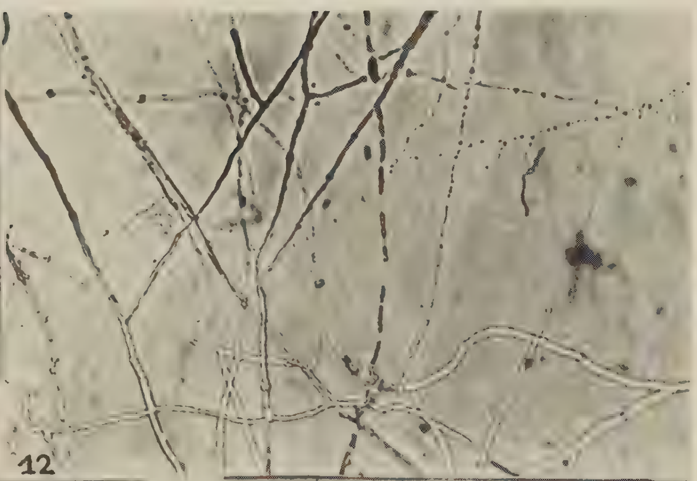
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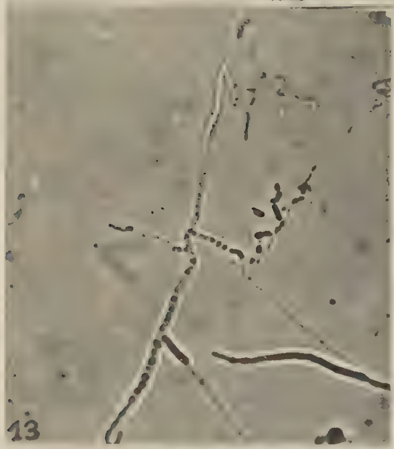
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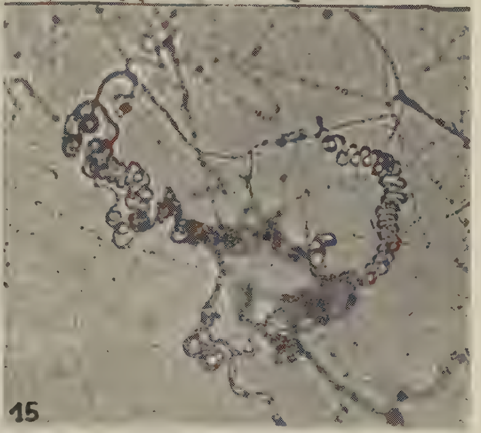
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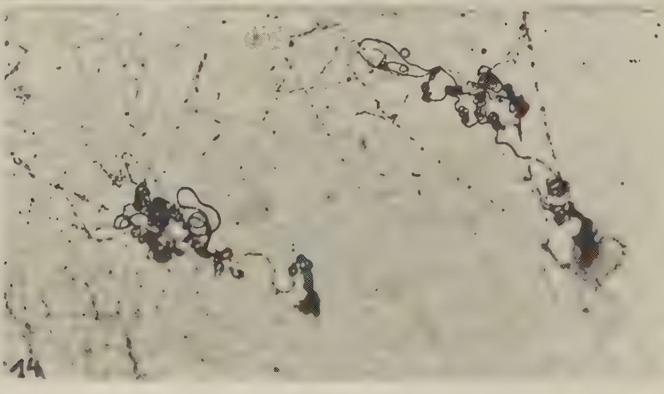
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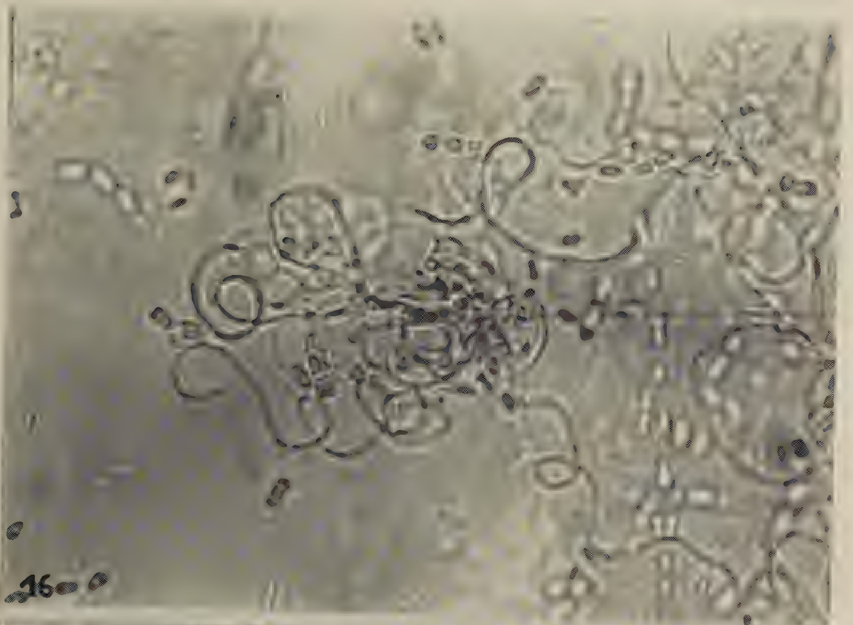

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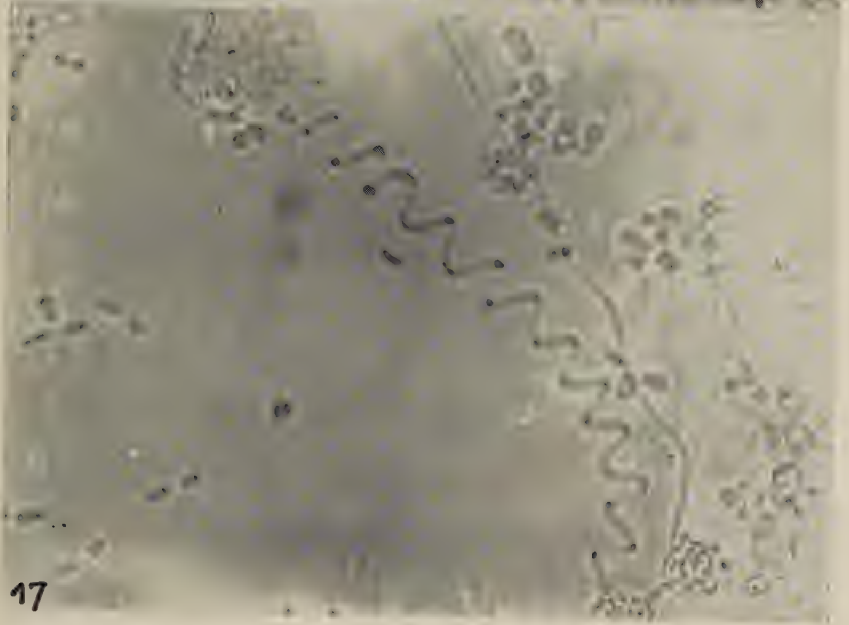

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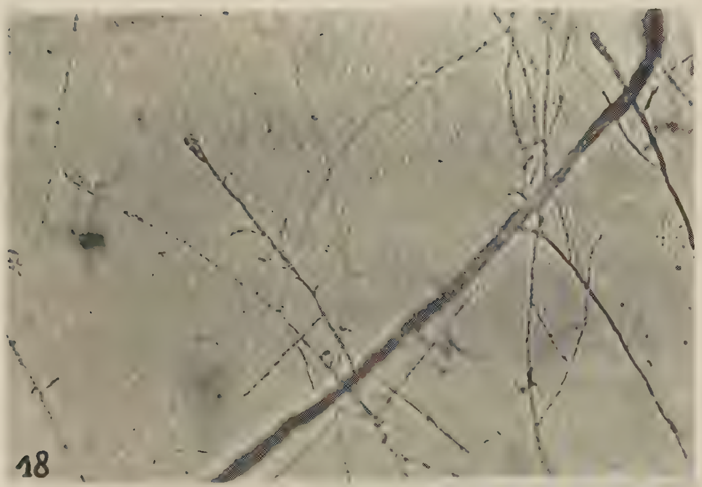

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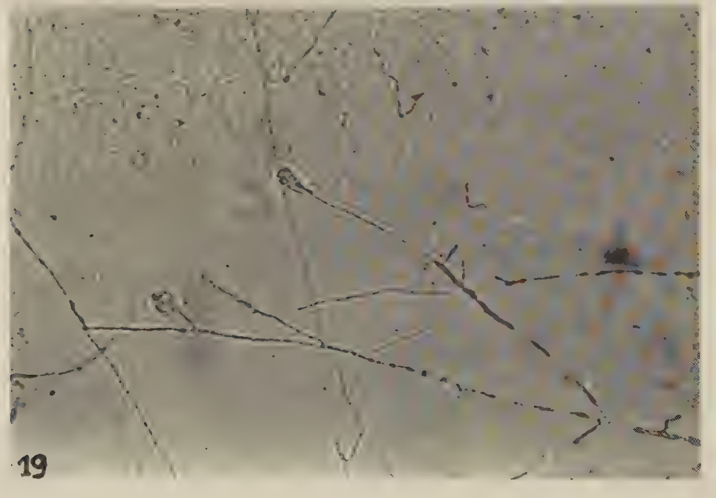

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n⁰ 5, 24 days old. Culture with aerial mycelium not very well developed, showing a rather granulous and farinaceous surface, resembling the *Trichophyton* of the *Gipseum* group; *e*) Strain 6, 25 days old. Culture of mixed type: in the central portion it is prostrate; in the inferior portion it is cottonlike (as in *b*), and in the superior portion it is rather granulous and farinaceous (as in *c*).

NOTE: In cornmeal agar the most common types are *a* and *b*.

In Sabouraud's glicosed medium and in the potato-agar media the macroscopic aspects are completely different for the aerial mycelium and its growth along the wall of the tube are much more abundant. When young, the cultures show soft cottonlike aspect; older cultures show a farinaceous somewhat pigmented aspect. The colour of the surface can be compared to pale pinkish buff (Ridgway, Pl. XXIX, 17″. O—Y. f) and in reverse they are sepia (Ridgway, Pl. XXIX, 17″. O—Y. m). Later on, in the old cultures, parts of the micelium break off and fall to the bottom of the tube, looking like a thick flour.

Fig. 2. Strain n⁰ 2, cornmeal-agar, 75 days old. Dissociated in lactophenol, between cover glass and slide. Central aleuriae of uniform size. Grapelike aleurophore; the membrane of the sterile portions of the hyphae which unites the aleuriae is well visible. In upper and lower left of the microphoto, spirals. × 700.

Fig. 3. Strain n⁰ 5. Photographed directly from the culture tube sent by Dr. N. F. Connant. Seriated central aleuriae. Sterile segments of the hyphae of various sizes joining the aleuriae together, and thereby showing that the fructification cannot be considered as an arthrospore. × 100.

Fig. 4. Strain n⁰ 7, cornmeal-agar, 56 days old. Central, irregular, seriated aleuriae; few lateral ones. Non differentiated protoplasmatic condensations. Microphoto obtained directly through the glass of culture-tube. × 30.

Fig. 5. Strain n⁰ 5, potato-agar-destrose, 46 days old. Like figure above. Vegetative hyphae, still not differentiated and branched. × 40.

Fig. 6. Strain n⁰ 7, cornmeal-agar, 54 days old. Central, seriated aleuriae. Lateral, pediculated aleuriae. Non differentiated vegetative hyphae. Segment of sterile hypha. Microphoto obtained directly through the glass of culture-tube. × 50.

Fig. 7. Strain n⁰ 7, cornmeal-agar, 54 days old. Sterile septated hyphae and vegetative hyphae in differentiation. Some aleuriae already differentiated. Microphoto obtained directly through the glass of culture-tube. × 50.

Fig. 8. Strain n⁰ 6, cornmeal-agar, 56 days old. Vegetative hyphae branching at almost right angles and bifurcated hyphae. Beginning of differentiation of aleuriae. Microphoto obtained directly through the glass of culture-tube. × 50.

Fig. 9. Strain n⁰ 3, cornmeal-agar, 58 days old. Differentiating aleuriae. Vegetative hyphae. Sterile septated hyphae. Spirals. Microphoto obtained directly through the glass of culture-tube. × 30.

Fig. 10. Strain n⁰ 8, photographed directly from tube sent by Dr. N. F. Connant. Central and lateral aleuriae. × 100.

Fig. 11. Strain n⁰ 8. Microphoto obtained directly through the glass of culture-tube. × 100.

Fig. 12. Strain n⁰ 6, potato-agar-destrose, 42 days old. Vegetative hyphae, some with and some without differentiation of the aleuriae. Septated and sterile hyphae. Microphoto obtained directly through the glass of culture-tube. × 75.

Fig. 13. Strain n⁰ 6, potato-agar-destrose, 42 days old. Vegetative hyphae. The central one already has aleuriae in differentiation. Microphoto obtained directly through the glass of culture-tube. × 40.

Fig. 14. Strain n⁰ 3, cornmeal-agar, 58 days old. Spirals. Sterile hyphae. Microphoto obtained directly through the glass of culture-tube. × 30.

Fig. 15. Strain n⁰ 3, cornmeal-agar, 58 days old. Spirals. Microphoto obtained directly through the glass of culture-tube. × 75.

Fig. 16. Strain n⁰ 2, cornmeal-agar, 75 days old. Dissociated in lactophenol between cover glass and slide. Spirals. Numerous aleuriae out of focus. × 150.

Fig. 17. Strain n⁰ 2, cornmeal-agar, 75 days old. Dissociated in lactophenol, between cover glass and slide. Spirals; one soft, another thick. Out of focus: aleuriae.

Fig. 18. Strain n⁰ 6, cornmeal-agar, 54 days old. Terminal mycelium sprouts. Vegetative hyphae and aleuriae already differentiated. Microphoto obtained directly through the glass of culture-tube. × 50.

Fig. 19. Strain n⁰ 6, cornmeal-agar, 54 days old. In the central zone of the microphoto, a terminal sprout. Bottom left, short mycelium ramification with sprouting; thin membrane. Cannot be considered chlamidospore: see Pl. 3, n⁰ 4 (chlamidospore) and Pl. 4, n⁰ 10, 11, 12, 13 (terminal mycelium sprouts). × 50.

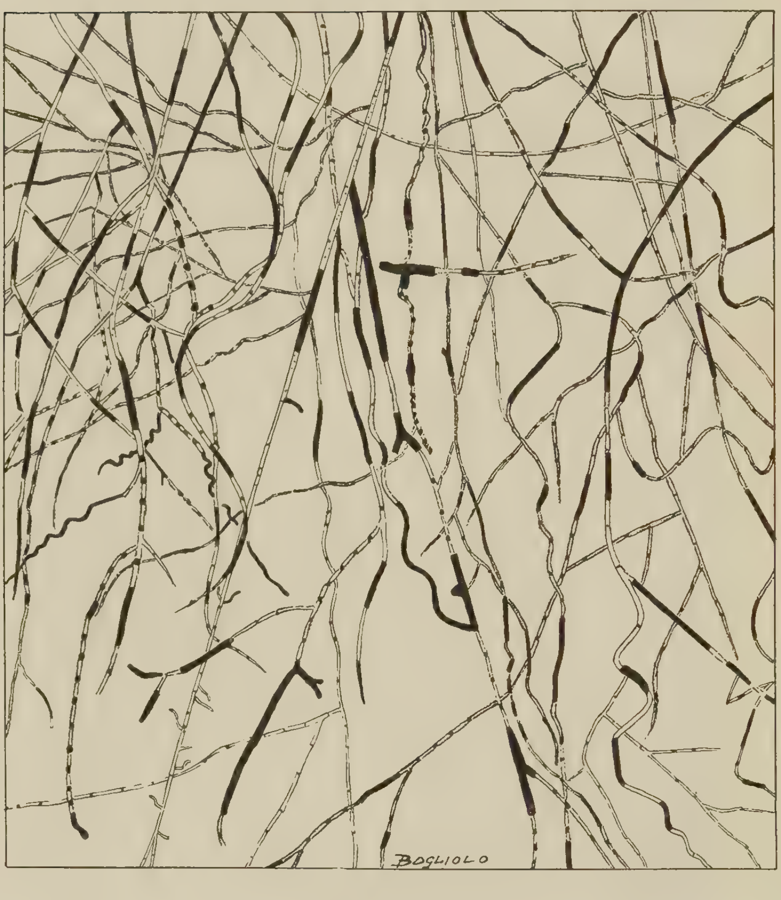

PLATE I

Beginning of the process of protoplasmatic condensation. Formation of soft spirals. Sterile hypha with irregular septation. Aleurias not yet differentiated. In this phase any micological diagnosis is not possible.
Strain n⁰ 5, 35 days old, cornmeal agar, reproduced directly through the culture tube. × 120.

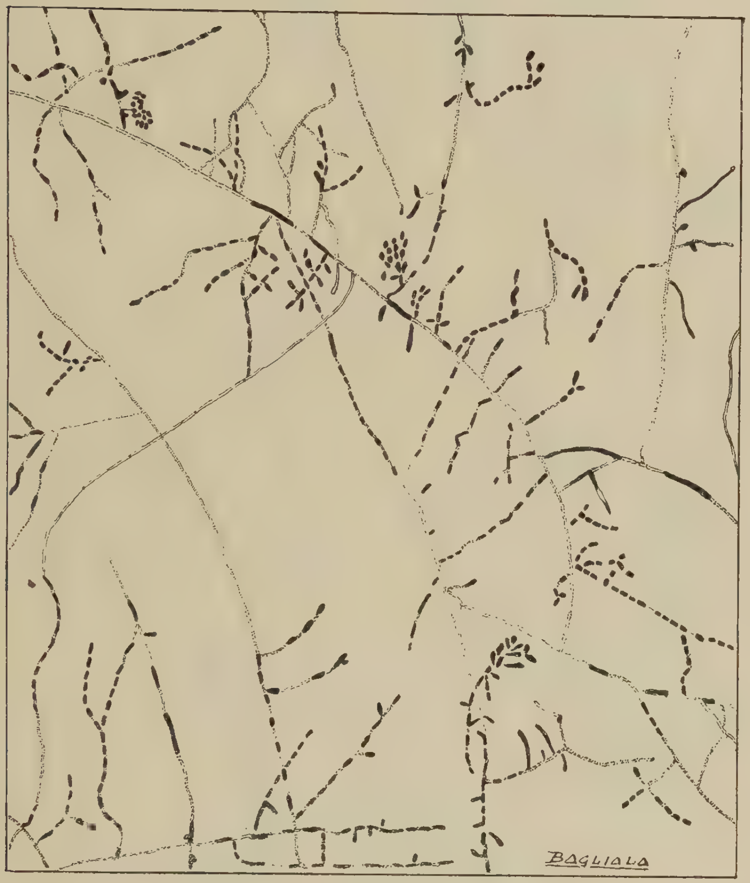

PLATE II

Aleuriae already differentiated. In some hyphae they are in series, central and of more of less constant dimensions; in others they are more irregular. Aleurospores with thyrsi and bunches. Persistence of non differentiated protoplasmatic reserves. Some sterile hyphae.
The same strain (n⁰ 5) of the Plate n⁰ 1, 59 day old, cornmeal agar, reproduced directly through the culture tube. × 90.

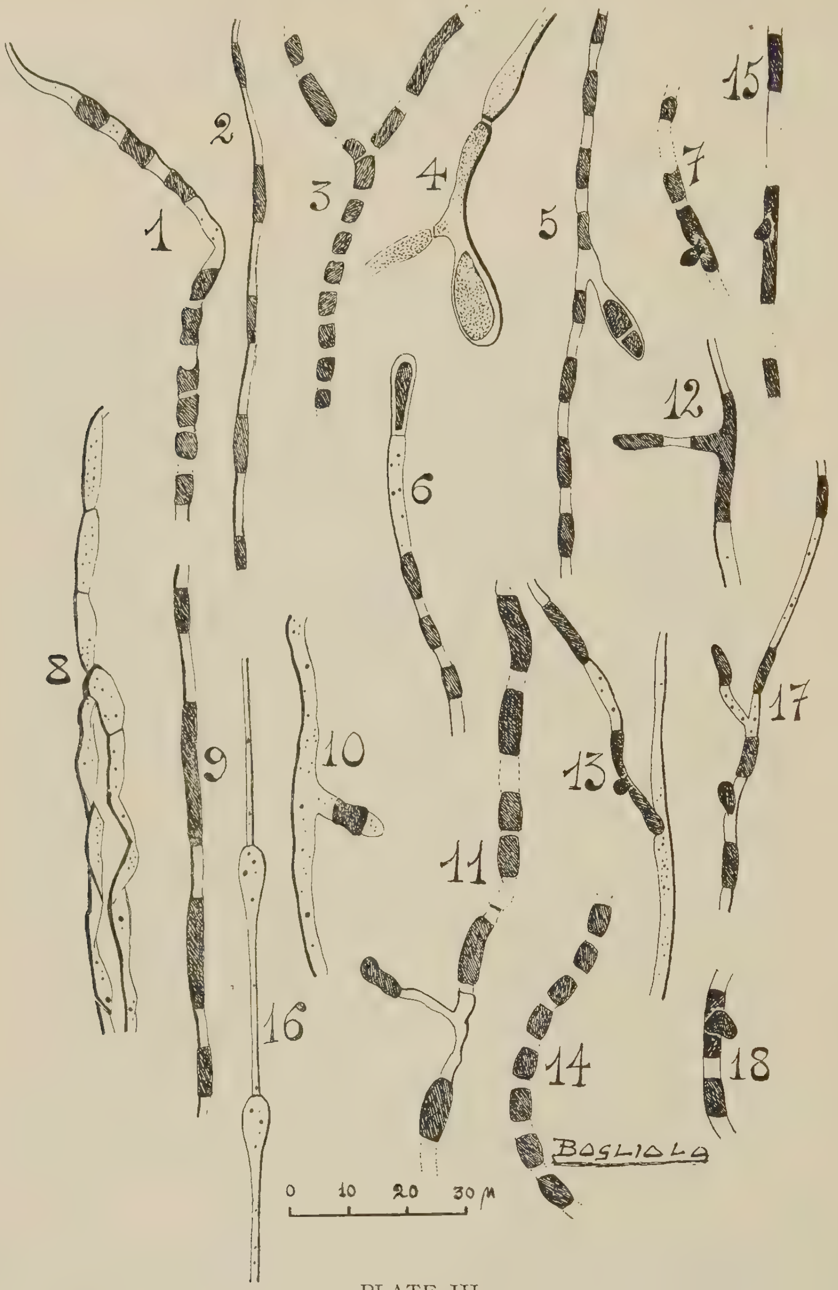

PLATE III

No 1. Central aleuriae, with sides which are either straight, concave or convex. Sterile spaces of different lengths. On some, drops of lipides.

No 2 and 9. Central aleuriae of different sizes. Sterile spaces of various dimensions.

No 3 and 14. Quite regular aleuriae. Sterile spaces regular too.

No 4. Formations like chlamidospores.

No 5, 11, 12 and 17. Hyphae with central aleuriae, with ramifications giving origen to other aleuriae. In 5, fusiform uniseptate aleuria, like those found in the *Achorion gallinae*. In 17, a non pediculated lateral aleuria, originating from a sterile part of the hyphae. Lipid drops in the sterile portions of the hypha.

No 6. Central aleuriae; a terminal one separated from the ones in series by a long portion of sterile hypha containing lipid droplets.

No 7, 13, 15 and 18. Lateral aleuriae originating from central ones.

No 8. Sterile irregularly septate, bifurcated hypha, containing lipid droplets. Probably represents a suffering stage.

No 10. Aleuria differentiated into a short ramification of sterile hypha.

No 16. Sterile hyphae in form of racket.

NOTE N⁰ 1 to 6 were obtained from strain n⁰ 5, from a 25-days old culture in cornmeal agar, dissociated in lactophenol between slide and coverslip. N⁰ 7 to 18, strain n⁰ 6, from 25-days old culture in cornmeal agar, dissociated in lactophenol between slide and coverslip. × 300.

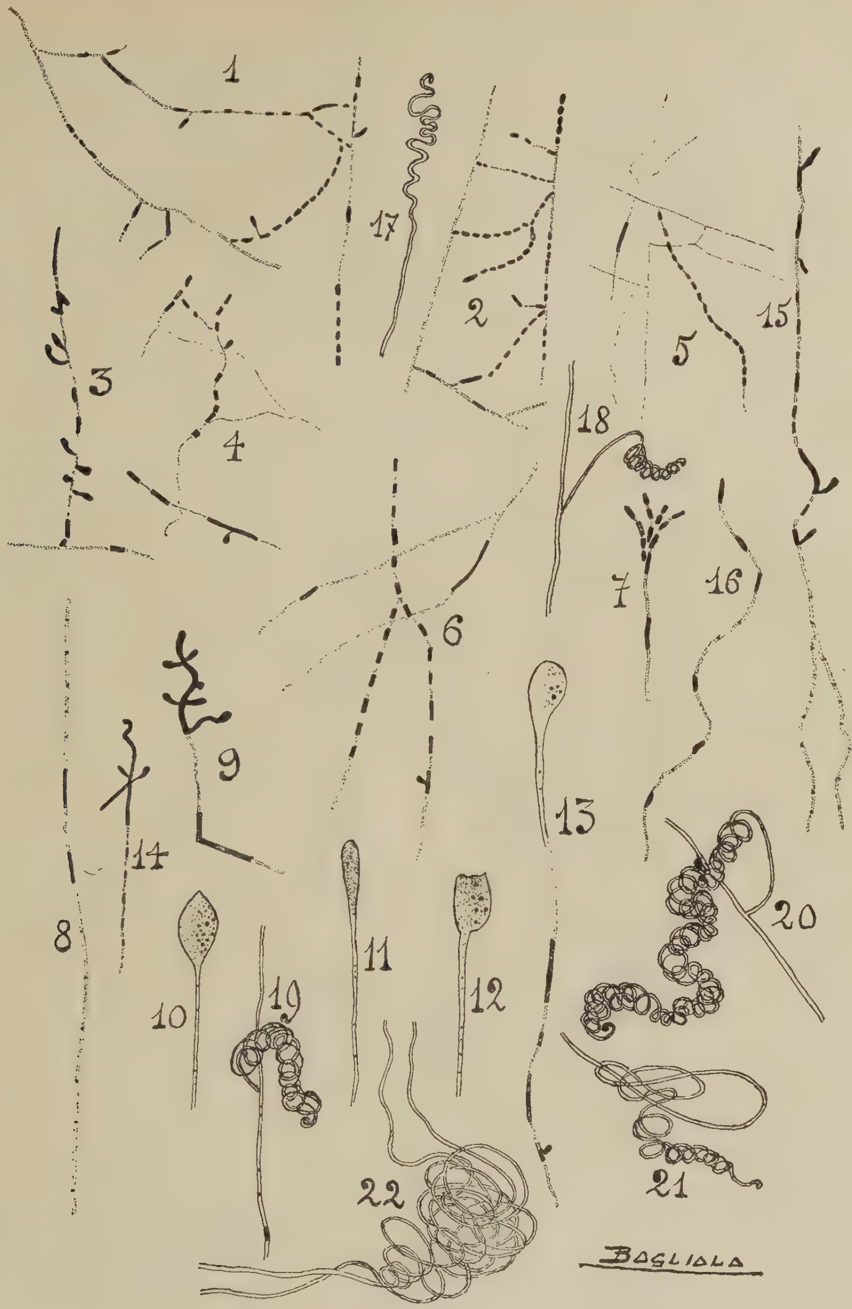

PLATE IV

No 1, 2, 4, 5 and 6. Sterile and vegetative hyphae, anastomosed or superposed. Central and seriated aleuriae. In 1, 4 and 6, lateral aleuriae with short pedicle. In 4, 5 and 6, citoplasmatic non differenciated reserves.
No 3. Not very differentiated aleurospores, with central and lateral aleuriae deriving from a sterile portion of a hypha.
No 7. Aleurophore, elementary, bunch-like.
No 8. Sterile hypha with citoplasmatic condensations well differentiated in aleuriae.
No 9 and 14. Terminal portions of hyphae with ramified citoplasmatic condensations, not well differentiated.
No 10, 11, 12 and 13. Irregular terminal mycelial dilatations, by some authors considered as terminal chlamidospores (see figures 17 and 18).
No 15. Central aleuriae; irregular and ramified citoplasmatic condensations.
No 16. Central and terminal aleuriae, separated by irregular portions of sterile hyphae (compare with nº 6 of Plate nº 3).
No 17, 18, 19, 20 and 21. Several types of spirals. No 22. Two intertwined spirals.
NOTE. Nº 1 to nº 3 were obtained from strain nº 5, from a 44 days old culture in cornmeal agar, dissociated in lactophenol between slide and coverslip. Nº 4 and 5 from strain nº 7, and nº 6 to nº 16 from strain nº 6; both, from a 56 days old culture in cornmeal agar, dissociated in lactophenol between slide and coverslip. Nº 17 to nº 22 were obtained from strain nº 3, from a 93 days old culture in cornmeal agar. Dissociated in lactophenol × 60.

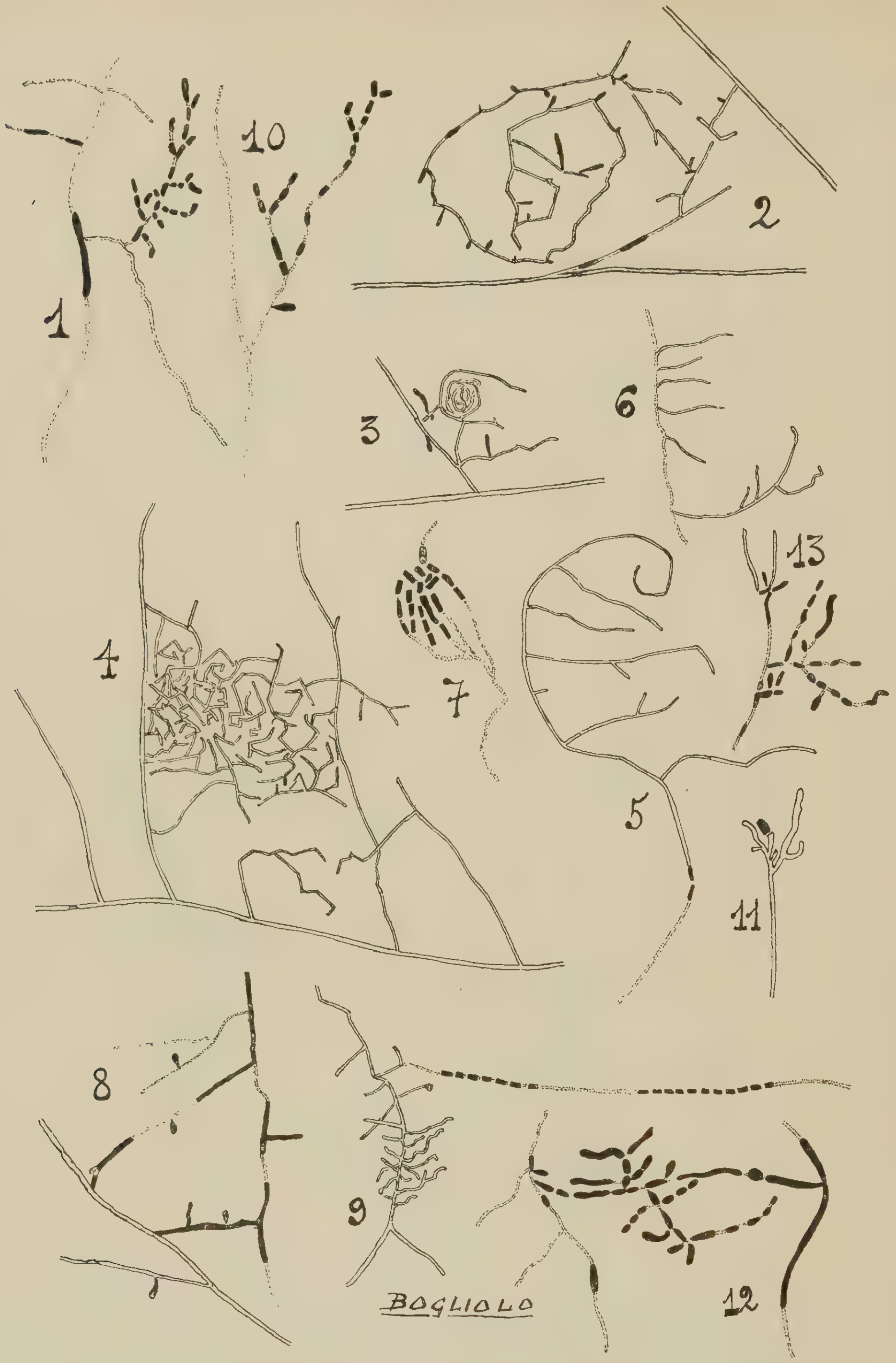

PLATE V

No 2. Portion of hyphae contracted with irregular and lateral aleuriae.

No 3. Soft spiral and lateral aleuriae.

No 4. Anastomoses or superpositions forming mass of sterile hyphae doubled in various angles, forming labyrinth-like.

No 5 and 6. Ramified mycelial formations like pectineous organs of *Achorion Schoenleini*.

No 8 and 12. Anastomoses or superpositions between sterile and vegetative hyphae, with irregular differentiation of central and lateral aleuriae.

No 9. Branching of sterile hyphae like branching observed in *Microsporum Audouini*.

No 11. Mycelial terminations like candelabra.

No 1, 7, 10 and 13. Various forms of aleurospores forming more or less differentiated bunches.

NOTE. N^0 1 and n^0 12 were obtained from strain n^0 8, from a 47 days old culture in cornmeal agar. N^0 2 to n^0 11 from strain n^0 7, from a 56 days old culture in cornmeal agar. N^0 13 from strain n^0 6, from a 48 days old culture in potato-agar-dextrose. Dissociated in lactophenol between slide and coverslip. × 120.

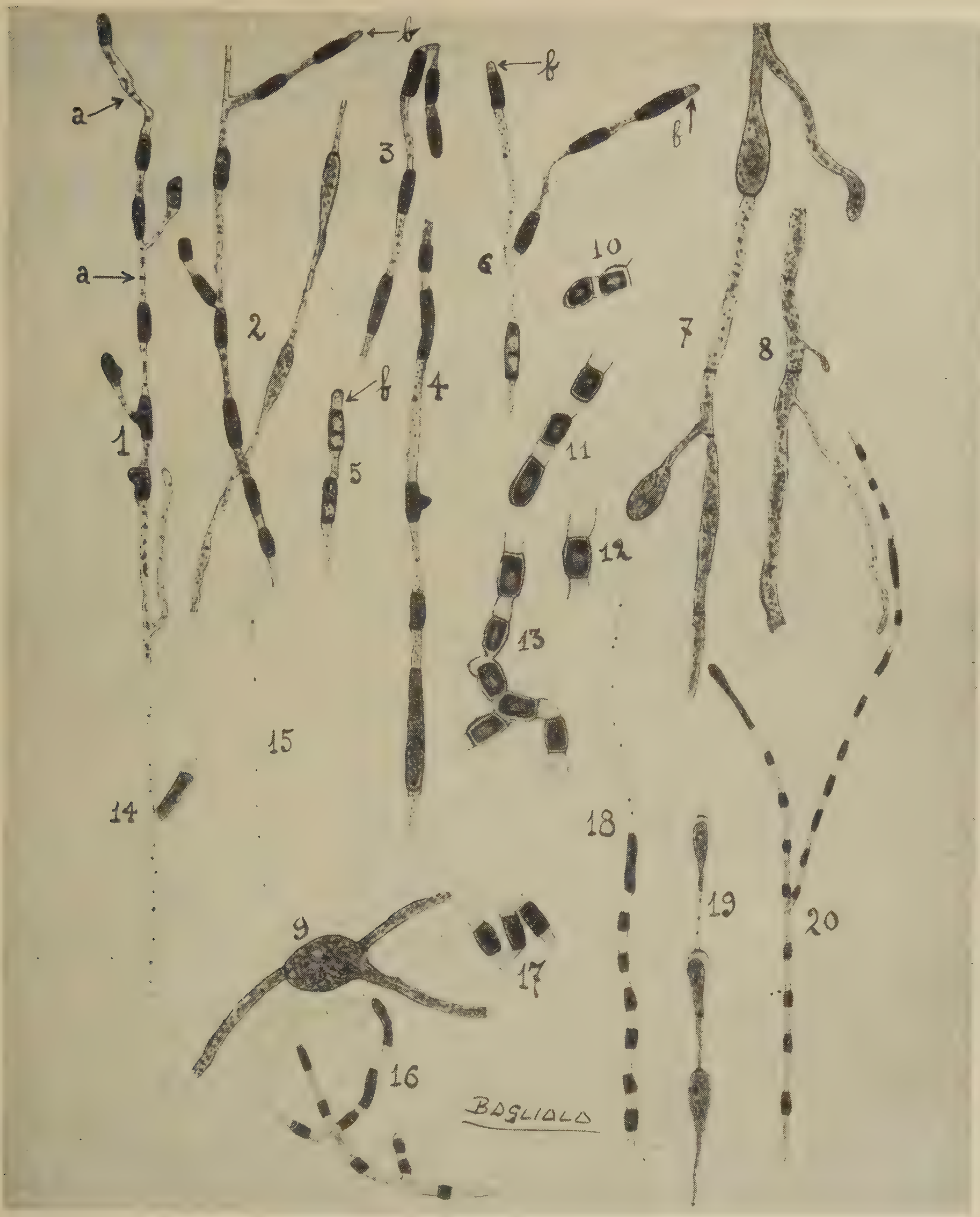

PLATE VI

No 1, 2, 3, 4, 5, 6, 10, 11, 12, 16, 17, 18 and 20. Central (sometimes seriate), lateral and terminal aleuriae, separated by some irregular and some regular portions of sterile hypha. In 1, *a*, see the septs in the sterile portions of hypha. In 2, 5, 6 *b* note that aleuria is separated from the terminal segment of the hypha by a short sterile length. 12: an aleuria separated from the others (possibily due to manipulations during dissociation); note both its ends continued by a fragment of sterile hypha. It was not separation of the aleuria, but rupture of sterile portions of the hypha which united this with other aleuriae. The same phenomenon is shown in n⁰ 10, 11, 13 and 17.

In 2, an aleuriphera hypha crosses a sterile one with racket formations.

17: aleuriae. The sterile portions of hyphae which unite the aleuriae are doubled-up (artefact due to manipulation).

No 7 and 19. Racket-like hyphae. In 7, two septs. No 8 and 15. Sterile hiphae. In 8, septation.

No 14. A non pediculated lateral aleuria, originating in a sterile portion of the hypha.

NOTE. N⁰ 1 to n⁰ 9 were obtained from strain n⁰ 5, from a 27 days old culture in cornmeal agar. N⁰ 10 to n⁰ 13, from strain n⁰ 3, from a 44 days old culture in cornmeal in potato-agar-dextrose. N⁰ 14 to 20, from strain n⁰ 5, from a 27 days old culture in potato-agar-dextrose. All, dissociated in lactophenol between slide and coverslip and stained with cotton-blue, under the action of slight heat which must, obviously, have altered the intimate structure of the aleuriae. For that reason, the structures represented in this Plate cannot be used as basis for citological study. × 300.

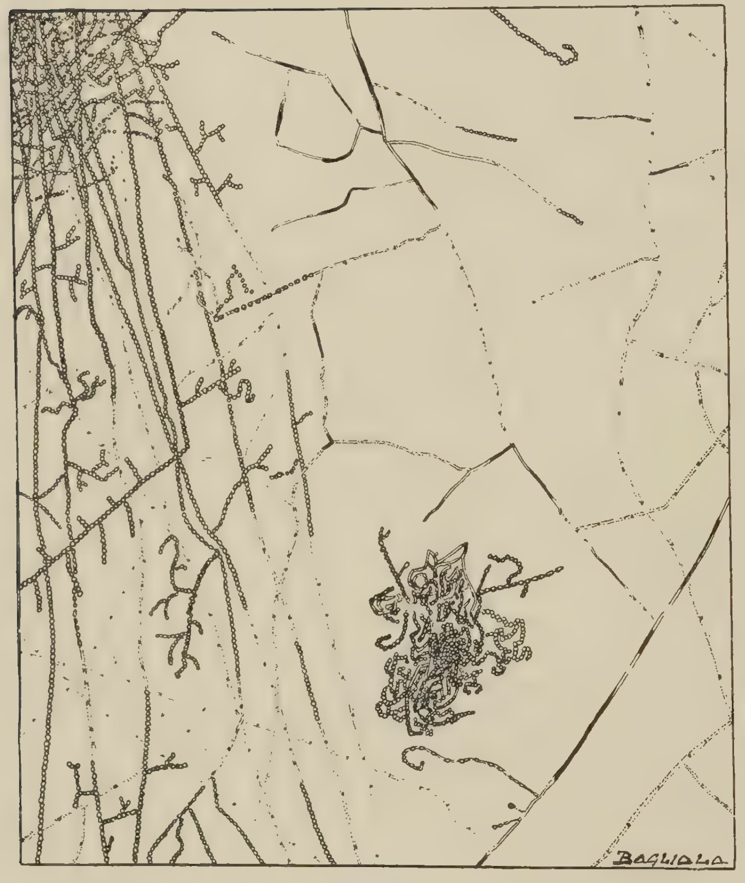

PLATE VII

Sterile hyphae with residues of protoplasmatic condensation. Vegetative hyphae with dehydrated aleuriae, contracted and with sterile portions between aleurias equally dehydrated and contracted. The lot simulate the *Scopulariopsis* form. Fragments of sterile and vegetative hyphae forming an irregular accumulation.
Strain nº 2, 753 days old. Sabouraud-glucose, directly through the tube of culture. × 120.

Est-il possible d'améliorer la nomenclature et la classification des dermatophytes.

par

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Les dermatophytes sont des champignons pathogènes ayant une affinité marquée pour la kératine: les couches cornées de l'épiderme, les cheveux, les poils, les ongles sont leurs points d'attaque et leur dermatophilie est un fait bien démontré. Botaniquement, ce sont des hyphomycètes dont les rapprochements — mais non l'identité — avec les gymnoascales ont été étudiés notamment dans les travaux de Matruchot et Dassonville. En taut que végétaux, ils tombent sous les règles de la nomenclature binaire et doivent être désignés par deux mots dont le premier indique le nom du genre, l'autre le nom de l'espèce.

Rien n'est plus évident que la confusion extrême de la nomenclature des dermatophytes. Un parasite aussi banal que le *Microsporum canis* est souvent désigné sous le nom de *M. lanosum* ou *M. felineum* ou encore, si l'on adhère à la classification de Langeron et Milochevitch,: *Sabouraudites canis, S. felineum* ou *S. lanosum*. A. E. de Area Leão et Masao Goto (1950) attribuent au *Trichophyton concentricum* Blanchard 1896 les synonymes suivants:

Trichophyton sp. Nieuwenhuis 1896
Lepidophyton sp. Tribondeau 1899
Lepidophyton concentricum Gedoelst 1902
Aspergillus lepidophyton Pinoy 1903
Trichophyton mansoni Castellani 1905
Trichophyton Castellani (Perry 1907) Castellani 1908
Endodermophyton concentricum Castellani 1910
Endodermophyton indicum Castellani 1911
Oospora conzentrica Hanawa et Nagai 1917
Arthrospora tropicalis Grigoraki 1925
Arthrospora indica Grigoraki 1925
Endodermophyton roquettei Vuillemin 1929
Mycoderma concentricum Vuillemin 1929
Achorion concentricum Guiart et Grigoraki 1928
Achorion indicum Guiart et Grigoraki 1928

C'est beaucoup et pourtant la liste n'est pas complète. Nous n'ignorons pas que beaucoup de ces synonymes sont tombés en désuétude, mais il n'en va va pas ainsi pour d'autres dermatophytes, par exemple pour le *Trichophyton rubrum* dont M. Ota et S. Kawatzure (1933) avaient, il y a longtemps déjà, reconnu 15 d'entr'eux.

Nous n'ignorons pas non plus que ceux qui ont l'usage de la nomenclature assimilent automatiquement un *Microsporum lanosum* ou un *Microsporum*

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[1]) Cette étude a été effectuée avec l'aide et un subside de l'Institut pour la Recherche Scientifique en Afrique Centrale (I.R.S.A.C.)

felineum au binome *M. canis* ou *Sabouraudites canis* qui a leur préférence mais il n'en reste pas moins qu'une confusion considérable règne dans la littérature et rend la lecture des travaux de mycologie médicale impossible aux non initiés. Pour les initiés eux-mêmes, la tâche est, dans certain cas, particulièrement ardue. S'agit-il par exemple d'établir des statistiques sur la répartition mondiale de telle ou telle mycose, on peut se demander jusqu'à quel point l'auteur a l'autorité de transformer *Microsporum felineum* en *M. canis* ou *Epidermophyton purpureum* en *Trichophyton rubrum* et s'il ne le fait pas son travail devient illisible, ses conclusions paraissent hasardeuses.

Basée sur une nomenclature aussi confuse, l'identification des dermatophytes reste chose compliquée et la propension, toujours trop marquée, à créer de nouvelles espèces, s'accuse. Selon nous, l'identification des dermatophytes est rendue difficile pour quatre raisons principales:

1°) Il existe plusieurs systèmes de classification. Cette difficulté n'est pas particulière à la mycologie et on la retrouve dans d'autres sciences qu'il s'agisse de classer un mollusque, un helminthe ou un insecte. Il sera d'ailleurs exceptionnel qu'ayant fait une bonne étude de la morphologie microscopique d'une culture d'un dermatophyte et de la lésion pilaire qu'il produit — ou ne produit pas — l'auteur ne parvienne à le faire entrer dans le système de classification qu'il affectionne.

2°) Les synonymies extrêmement nombreuses constituent le second obstacle à une classification simple.

3°) Le polymorphisme des dermatophytes représente une troisième difficulté. Ce polymorphisme peut apparaître dans les primocultures et dans les subcultures. Une souche atypique d'un dermatophyte bien connu peut ainsi jouir du crédit d'une espèce nouvelle. VANBREUSEGHEM étudiant 136 souches de *Trichophyton ferrugineum* (1950) (que beaucoup appellent *Microsporum ferrugineum* en raison de la lésion de type microsporique qu'il fait apparaître dans le cheveu) a pu distinguer à côté de la forme rouille classique, une variété blanche; de plus, il a démontré que chacune de ces variétés pouvait présenter 4 types différents de colonies qu'il était possible d'isoler séparément ou simultanément d'un même produit pathologique, et qui pouvaient se transformer l'un dans l'autre. Si l'on considère qu'un de ces types était représenté par une colonie duveteuse, un autre par une colonie rappelant étroitement le *Trichophyton* (*Achorion*) *schonleini* on doit admettre que l'identification de certains dermatophytes est chose particulièrement difficile.

4°) Le pléomorphisme des dermatophytes, phénomène de transformation irréversible de la souche initiale qui progressivement se couvre d'un duvet blanc constitué de filaments mycéliens minces et stériles, conduit le dermatophyte à une morphologie si différente de celle de la primoculture que Sabouraud, qui fut le premier à le décrire, le prit longtemps pour une contamination ou, comme il écrivait, pour un phénomène de commensalisme. La nature de cette transformation nous est encore inconnue: nous ne connaissons que quelques-unes des conditions de son apparition, notamment la culture sur les milieux hypersucrés. Certains auteurs considèrent le pléomorphisme comme l'apanage de certaines espèces, d'autres comme un phénomène général et on a été amené à reconnaître à côté de la forme duveteuse blanche classique du pléomorphisme des formes atypiques qui n'en constituent pas moins des formes des dégénérescences irréversibles (cf. CATANEI 1929). Le pléomorphisme constitue le 4me. obstacle à une identification aisée des dermatophytes.

On nous dira que si un chercheur se trouve en présence d'une souche de dermatophyte qu'il ne connaît pas il peut la soumettre à un expert. Nous répondrons que cet expert n'existe probablement pas pour la raison que les dermatophytes sont répartis de façons très particulière suivant les régions géographiques et que le spectre des dermatophytes d'une région n'est pas celui d'une autre. En somme, chaque spécialiste des dermatophytes n'en connaît bien qu'un certain nombre d'espèces. On pourra également nous objecter que ce chercheur pourrait fort simplement s'adresser à un centre mycologique possédant de vastes collections et demander la souche ou les souches qui paraissent correspondre, d'après les descriptions qu'il en a lues, à la souche qu'il a isolée. Hélas, beaucoup de souches sont dégénérées et les centres mycologiques ne pourront envoyer au chercheur que ce qu'ils possèdent. Supposons d'autre part qu'un chercheur croie avoir isolé le *Trichophyton soudanense* décrit en 1912 par Joyeux; il lui sera impossible de comparer cette souche à la culture initiale qui, si elle existe encore, a complètement changé d'aspect. Pour notre part, ayant isolé du *T. soudanense* au Congo Belge (1950) nous n'avons pu arriver à son identification — probable — que par une série de recoupements dont la valeur n'est pas celle que pourrait avoir une comparaison à un étalon.

Classifications différentes, nomenclature confuse, polymorphisme et pléomorphisme, tels sont les obstacles majeurs que trouve le chercheur sur son chemin. Alors que le malacologue, l'helminthologue, l'entomologiste, peuvent comparer les espèces qu'ils découvrent à des exemplaires bien conservés, le mycologue se voit contraint de comparer les souches qu'il isole à des descriptions livresques toujours fortement teintées d'éléments personnels, à des photographies toujours imparfaites, à des souches toujours mouvantes conservées dans des collections.

Existe-t-il un remède susceptible d'améliorer cet état de chose? Nous n'en voyons qu'un, d'application difficile sans doute, mais susceptible d'assainir nos idées dans un domaine où la confusion règne en maîtresse et de réduire les synonymes multiples. Il faudrait que ceux qui isolent des champignons des dermatophyties — ce nom, à l'exclusion de tout autre devrait désigner les affections causées par des dermatophytes — envoient les souches qu'ils ont isolées et dont l'identification n'est pas évidente, non pas à un laboratoire mais à 5 ou 10 laboratoires répartis dans le monde entier et désignés à cet effet par une autorité en raison de leur connaissance de la flore dermatophytique de la région dans laquelle ils travaillent. Chaque laboratoire ferait indépendamment des autres une identification dont les résultats seraient communiqués au chercheur soit directement soit par l'intermédiaire d'un bureau centralisant les résultats. Du conflit qui ne manquerait pas de naître assez souvent, finirait sans doute par jaillir quelque lumière. Ce remède que nous proposons est d'application difficile. C'est le seul qui nous paraisse actuellement avoir quelque chance de réussir.

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Carlo E. Malan

Sopra un interessante plectomicete umicolo nuovo per la scienza: *Peyronellula mirabilis* n.g. et n.sp.

(*Dall' Istituto di Patologia vegetale e Microbiologia Agraria e tecnica dell' Università di Torino*)

(1.XI.1951)

Durante un sopraluogo fatto ai primi di Ottobre 1948 in un campo sito nei dintorni di Pinerolo, il prof. Peyronel attirava la mia attenzione sopra estese placche d'un rosa pallido o bianchiccie che ricoprivano qua e là il terreno. Ad un superficiale esame macroscopico tali placche richiamavano alla mente quelle che sulle aree carbonarie, sul terreno sterilizzato in autoclave, talora sull'intonaco di locali umidi, produce il *Pyronema omphalodes* (Bull.) Fuckel, tanto più che alla lente esse risultavano costituite in parte di masserelle sferoidali, in parte di minute tazzette, le prime delle quali si poteva ritenere rappresentassero degli apoteci ancora chiusi, le seconde apoteci già aperti e in parte maturi. Senonché, prelevati campioni e fattone in laboratorio l'esame microscopico, si constatò trattarsi di un fungillo del tutto diverso e che, per le sue peculiari caratteristiche morfologiche, risultava quanto mai interessante. Il Prof. Peyronel me ne affidava lo studio, seguendomi e consigliandomi nelle ricerche, spesso ardue, mediante le quali potei ottenerne l'isolamento in coltura pura e fissarne il ciclo biologico, la morfologia e la sistematica.

Il fungo in natura

Il campo ove comparve il fungo in istudio era coltivato a viti, in filari distanziati, con colture erbacee negli interfilari. Nell'autunno del 1947 era stato seminato del grano (previa concimazione a base di perfosfato), al quale nel marzo 1948 era stato consociato del trifoglio pratense. Dopo la mietitura, avvenuta nella seconda metà di giugno, il trifoglio fu lasciato indisturbato fino ad agosto, quindi tagliato ed il terreno rovesciato, erpicato e rullato. Ai primi di ottobre, al momento del sopraluogo, il trifoglio era sporadicamente ricomparso, lasciando però nudi discreti tratti di terreno compresso e liscio, sui quali appunto larghe chiazze crostose di color rosa pallido rivelavano la presenza del fungillo. Questo si presentava sotto l'aspetto di numerosi puntini variamente raggruppati (Tav. XXXII Fig. 1) che, esaminati alla lente, risultavano essere delle masserelle rosee e quasi diafane, sferoidali, talora, negli aggregamenti più fitti, più o meno deformate per mutua compressione, di diametro variante fra i 300 e i 500μ circa, di consistenza carnosa.

Nei tratti ove il terreno era meno umido (la notte precedente c'era stata un'abbondante rugiada e la nebbia aveva ricoperta la zona fino verso le otto del mattino) le sferette fungine avevano perso un po' della loro turgescenza e presentavano una depressione al polo superiore (Tav. XXXII Fig. 2).

Un nuovo sopraluogo, effettualo dopo tre giorni di tempo bello e relativamente caldo, mise in evidenza gli effetti del progressivo disseccamento del fungo; al polo libero di ogni sferetta la depressione si era accentuata sino ad assumere l'aspetto di un cratere talora tanto profondo che le massoline venivano ad assumere l'aspetto di tazze o addirittura di anelli.

Nell'ultima decade di ottobre, dopo una successione di belle giornate, il fungillo sembrava scomparso e solo esaminando attentamente il suolo se ne scoprivano tenui tracce: sottili dischetti privi o quasi di colore che andavano vieppiù confondendosi colle particelle di terra. Non fu più possibile, nello scorcio dell'anno 1948, seguire ulteriormente le sorti del funghetto nel suo ambiente naturale perchè il giorno dopo l'ultimo sopraluogo il terreno veniva arato per essere sottoposto a nuove colture.

Nella stagione autunnale del 1949 il funghetto non comparve alla superficie del terreno sotto l'aspetto caratteristico assunto l'anno precedente, ma periodici esami microscopici del terreno ne rivelarono, sin dai primi di settembre, la presenza sotto forma, questa volta, di periteci isolati e completamente ipogei. Molte possono essere le cause della mancata comparsa della fase epigea del fungo, ma fra queste le principali sono probabilmente da ricercarsi nell'andamento stagionale, notevolmente meno ricco di umidità dell'anno precedente, e nel precoce sopraggiungere del freddo.

Aspetto microscopico

All'esame microscopico le masserelle sferoidali risultano formate d'un ifenchima stromatico cavo, la cui parete è costituita di innumerevoli periteci rotondeggianti immersi in un intreccio di ife sottili, piuttosto compatto, ma poco sviluppato — tanto che i periteci sono quasi a contatto tra di loro — e differenziato all'esterno in un rivestimento pseudoparenchimatico di poco spessore, ricoperto negli esemplari meno evoluti di una tenue lanuggine fioccosa. La cavità delle sferette stromatiche non è del tutto vuota, ma contiene un intreccio assai lasso di ife.

I fiocchetti che rivestono gli stromi risultano costituiti di ife sottili riunite in sinnemi o cordoncini sui quali sono inseriti, in numero non rilevante, conidiofori jalini, semplici, foggiati a bottiglia allungata o a manico di frusta, misuranti 30—50μ di lunghezza per 2—3 di diametro e recanti alla loro estremità un glomerulo di conidi jalini, oblunghi o subcilindrici, continui, spesso 1—2 guttulati, 6 — 10 × 2 — 3 μ.

I periteci (Fig. 1) contenuti nella parete stromatica sono, com s'è detto, globosi ed a maturità misurano da 80 a 120 μ di diametro; essi sono assolutamente astomi e rivestiti di un eccipulo o peridio pseudoparenchimatico dello spessore di 10—20 μ con pseudocellule che, viste dall'esterno, presentavano un contorno ondulato.

Il contenuto dei periteci immaturi è costituito di una massa granulosa indistinta nella quale si differenziano successivamente delle massoline sferoidali, in numero variabile a seconda della grandezza del peritecio, rivestite di una sottile membrana, che rappresentano gli aschi. Questi misurano 20—24 μ di diametro. Negli aschi (Fig. 2) appaiono ben presto gli abbozzi di otto ascospore ellittiche, continue, inizialmente jaline, immerse in un epiplasma mucoso fortemente rifrangente che tenui sepimenti suddividono in otto parti (quante sono le ascospore) poliedriche per mutua compressione. Col proseguire della maturazione in ogni ascospora compare una grossa guttula oleosa centrale di diametro poco inferiore al diametro trasverso dell'ascospora stessa, ed intanto il muco si condensa in una pellicola di un bruno sempre più intenso fino al color caffé. La pellicola mucosa, che costituisce un otricello di dimensioni notevolmente maggiori di quelle dell'ascospora, aderisce in parte alla superficie di questa secondo settori fusiformi longitudinali, ed in parte forma delle pieghe, pure longitudinali, che separano l'uno dall'altro i settori di adesione. Di siffatte coste sporgenti se ne formano da tre a sei, per lo più 4—5.

Le ascospore (Fig. 3) perfettamente mature sono adunque di color caffé, hanno forma ellissoidale e sono percorse a guisa di meridiani da 3—6 coste o creste longitudinali confluenti alle loro estremità, e misurano 10 — 14 × 5—6,5 per lo più 11 × 6 μ.

Col maturare delle spore la membrana dell'asco si gelifica e scompare, lasciandole libere nella cavità periteciale.

Liberando delicatamente gli stromi dalle particelle terrose che ne circondano la parte inferiore, frequentemente si nota come essi si prolunghino inferiormente nell'interno di una specie di tubo che si va assottigliando e ramificando, d'un colore bruno-violaceo che contrasta nettamente con quello roseo degli stromi stessi. Un'indagine comparativa permette di affermare che siffatti tubi non sono che dei protonemi e relativi rizoidi di Muschi morti, nell'interno dei quali il fungo si è insinuato, formandovi non solo la parte inferiore, radiciforme, dei propri stromi, ma anche grossi periteci del tutto identici a quelli contenuti nella parte globoidale epigea. Ed infatti i muschi, per lo più ancora allo stato di protonemi, vegetavano abbondantemente nel terreno ove erano comparse le fruttificazioni stromatiche del fungo.

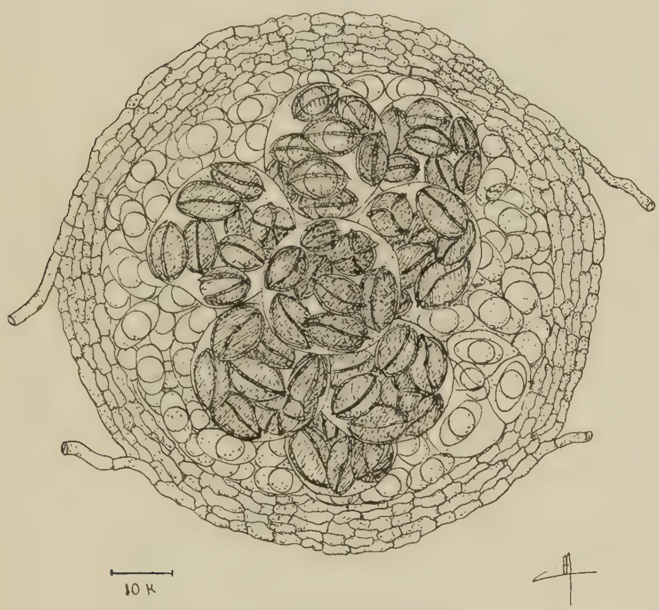

Fig. 1.
Peritecio di *Peyronellula mirabilis* (× 800)

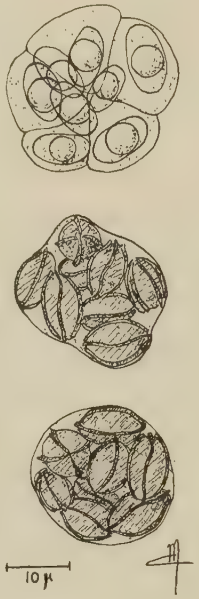

Fig. 2.
Aschi in vari stadi di maturazione (× 800).

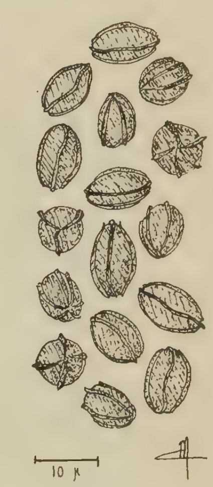

Fig. 3.
Ascospore (× 800).

Isolamento e coltura

L'isolamento del fungo in coltura pura non presenta notevoli difficoltà: già semplicemente trasportando dei pezzi del tessuto stromatico prelevati asetticamente dall'interno degli stromi — previamente liberati con un prolungato lavaggio, dalle particelle di terra incrostanti, — su agar-carote acidificato, ho ottenuto una discreta quantità di colture pure; risultati anche migliori ho avuto trasportando sul substrato nutritizio gli stromi interi previamente lavati in alcool e sciacquati in acqua sterile. Trasportando, poi, singole ascospore prelevate da periteci contenuti negli stromi raccolti in natura, — cosa relativamente facile date le loro dimensioni ed il loro colore — in tubi di coltura e su pezzetti di agar-carote compresi fra due vetrini, (microculture), ho potuto seguire l'intero ciclo di sviluppo del fungo dalla germinazione delle ascospore alla formazione dei nuovi corpi fruttiferi. La germinazione delle ascospore prodotte in natura ha luogo dopo 6—7 giorni su agar-carote acido, alla temperatura di 22°. Il meccanismo della germinazione è il seguente: prima di tutto il muco che s'era addensato a guisa di membrana attorno alla spora si rigonfia, la spora assume una forma sferoidale ed il suo colore impallidisce; successivamente ad una o ad ambe le estremità della spora, eventualmente anche a metà di essa, si producono dei tubi germinativi grossi 3,5—4 μ, i quali, allungandosi successivamente, si assottigliano fino a soli 2,5—3, si ramificano e si settano (Fig. 4). Seguendo lo sviluppo di queste prime ife in microcultura, se ne osserva il rapido irradiarsi in tutte le direzioni e il formarsi di numerose anastomosi che le congiungono in un reticolo di mano in mano più fitto. Dopo 36 ore circa compaiono qua e là sulle ife delle brevi ramificazioni incurvate ad uncino che poi si contorcono ad elica. Tali organi elicoidali rappresentano evidentemente dei gametangi, ed infatti, partendo da due ife distinte poco discoste od anche da due punti vicini d'una stessa ifa, si incurvano a due a due l'uno verso l'altro fino ad incontrarsi, si attorcigliano assieme, mentre altre ramificazioni proliferanti dalla loro base li avvolgono strettamente (Fig. 5): si formano così dei grovigli dap-

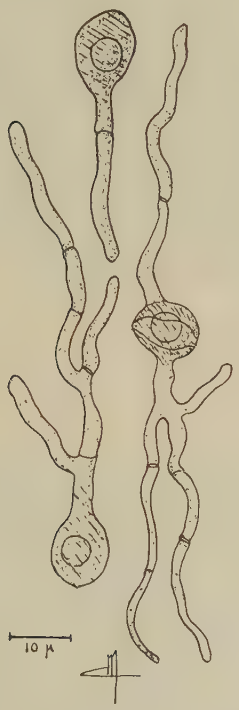

Fig. 4.
Ascospore germinanti (× 800).

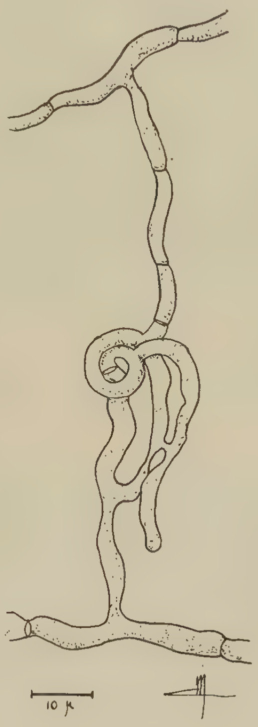

Fig. 5.
Primo abbozzo di un peritecio (× 800).

prima irregolari, poi di mano in mano assumenti forma sempre più regolarmente tondeggiante e aspetto di gomitoli nei quali, dopo 24 ore dalla comparsa dei gametangi, già sono ben riconoscibili degli abbozzi di periteci. Dopo 3—4 giorni vi si distinguono l'eccipulo, gli aschi e le ascospore (queste ancora jaline), il tutto costituente periteci perfettamente identici a quelli formantisi in pieno campo.

Quando la coltura, anziché dalle ascospore, ha origine da micelio asportato dallo stroma od ottenuto da una precedente coltura, la comparsa dei periteci è molto più precoce perchè lo sviluppo del fungo è abbreviato del periodo di 6—7 giorni necessario al rigonfiamento ed alla germinazione delle ascospore. Partendo da micelio, dunque, lo sviluppo del fungo in coltura nelle stesse condizioni in cui ho seguito dianzi quello delle ascospore, ha questo ritmo: per le prime 48 ore si ha uno sviluppo puramente vegetativo, le ife crescono, si ramificano, e si anastomizzano attivamente; al terzo giorno si verificano gli aggrovigliamenti di ife che preludono alla formazione dei periteci, dei quali, al quarto, già si distingue l'eccipulo, all'ottavo gli aschi, al nono le ascospore, che raggiungono l'aspetto definitivo all'undicesimo giorno dall'inizio della coltura.

Dei terreni messi in opera per lo studio dei caratteri colturali del fungo, (Tav. XXXII Fig. 3) l'agar-carote acido si è rivelato il più adatto al suo sviluppo ed alla produzione dei corpi fruttiferi; seguono, in ordine decrescente di efficacia, fra i terreni solidi: l'agar-carote neutro, l'agar-malto, i tasselli di carote, i culmi di segale, i tasselli di patata, lo Czapeck agarizzato; per quelli liquidi: il decotto di carote acido ed il liquido di Czapeck.

L'aspetto macroscopico delle colture su substrato solido varia a seconda che esista o no alla superficie del substrato stesso un velo di liquido. Sull'agar-carote acido non più freschissimo, dalla cui superficie già sia evaporato il velo di liquido che ricopre il substrato fresco, si ha, in 3—4 giorni, la produzione di una sola colonia nel punto ove si è depositato l'inoculo, rosea, rilevata e subito di aspetto filamentoso. Su substrato fresco, invece, nello stesso lasso di tempo e nelle stesse condizioni di temperatura, subito dopo l'apparizione della colonia sviluppatasi al punto di inoculazione, la superficie del substrato si dissemina di numerose coloniette circolari, rilevate, rosee, di aspetto mucido. Dopo 6—7 giorni, scomparso il velo liquido superficiale, anche queste coloniette, che, come vedremo, hanno origine da conidi, perdono l'aspetto mucido e diventano del tutto simili a quelle comparse sin dall'inizio sui substrati meno ricchi di umidità.

Lo sviluppo in estensione del fungo, anche nelle migliori condizioni ambientali, è lento; raramente, specie nelle colture in matraccio, il micelio riesce a coprire tutta la superficie del substrato; in compenso le ife formano un intreccio che si fa di mano in mano sempre più compatto tanto da giustificare la qualifica di stroma.

Dopo 10—11 giorni dall'inizio della coltura il colore roseo delle colonie scompare a cominciare, nei tubi di agar carota, dalla parte superiore del ,,becco di clarino" e viene sostituito dal grigio ferro impartito dalla massa di ascospore in via di maturazione prodottesi nei numerosi periteci venuti differenziandosi sotto il sottile, ma compatto velo di micelio roseo.

Nelle colture in matraccio la maturazione dei periteci ha inizio al centro della colonia e prosegue a zone concentriche dando alla colonia stessa l'aspetto di una coccarda ad anelli alternati grigi e rosei, con spiccati solchi radiali. Dopo circa un mese tutti i periteci sono maturi e la colonia ha assunto un colore grigio ferro con minuti puntini neri in quelle zone ove i periteci maturi sono maggiormente addensati.

Sull'agar di carote neutro, come su tasselli di carote, il fungo presenta caratteristiche colturali identiche a quelle rilevate per l'agar di carote acido. Su

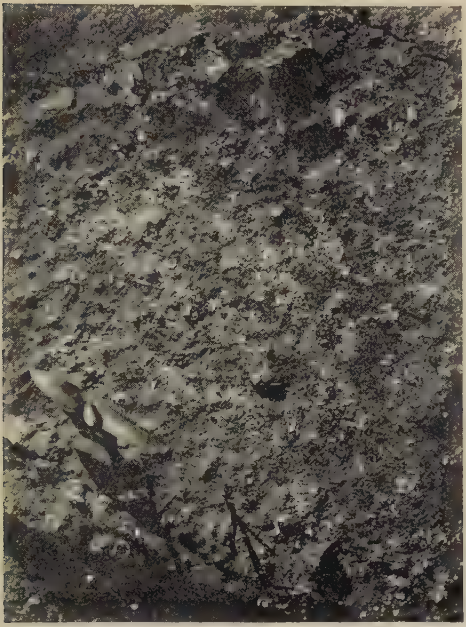

Fig. 1. Tratto di terreno ricoperto di stromi di *Peyronellula mirabilis* (circa ¼ delle dimensioni naturali).

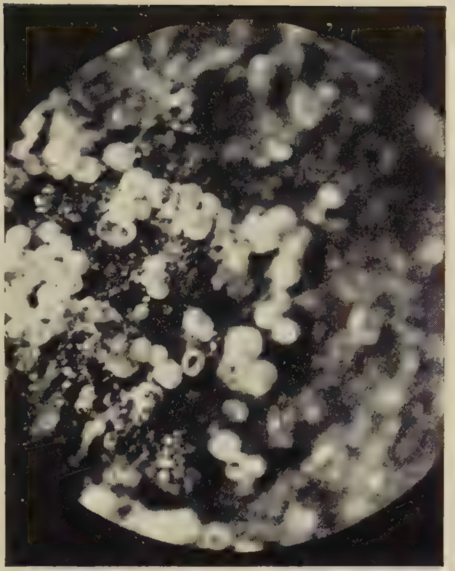

Fig. 2. Aspetto naturale degli stromi di *Peyronellula mirabilis* (× 10).

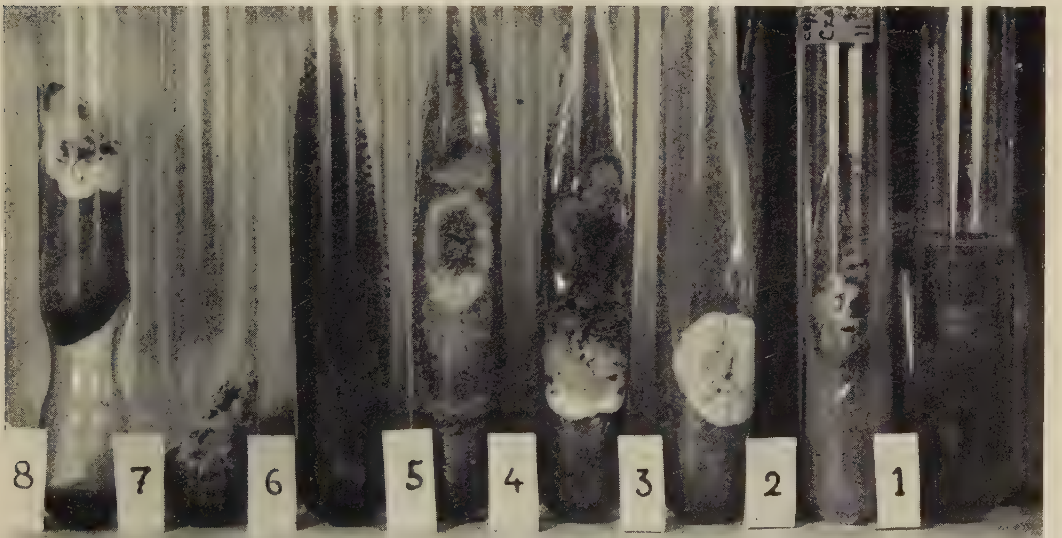

Fig. 3. Aspetto delle colture su: 1) liquido di Czapeck; 2) Czapeck agarizzato; su agar di carote acidificato dopo 35 giorni (3) e dopo 70 giorni (4), (5), (6); 7) su tassello di carote; 8) su tassello di patata.

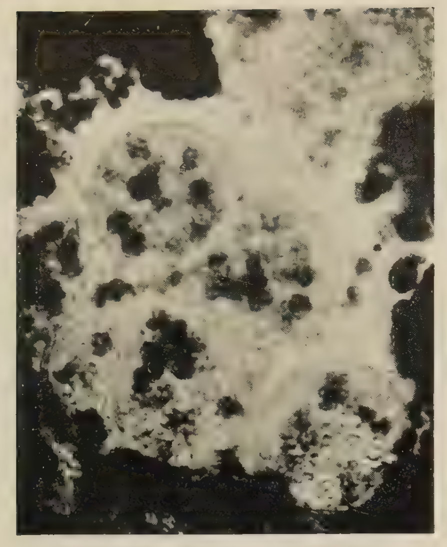

Fig. 1. Periteci di *Peyronellula mirabilis* in coltura (× 200 ca.).

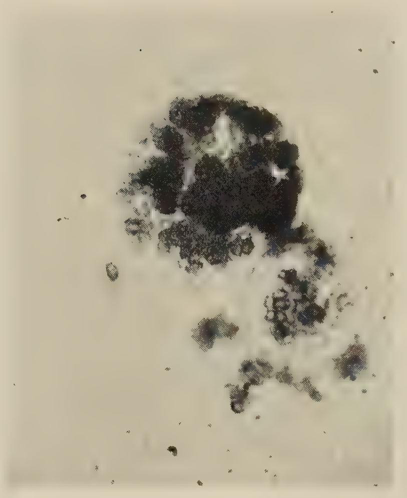

Fig. 2. Periteci maggiormente ingranditi (× 250 ca.).

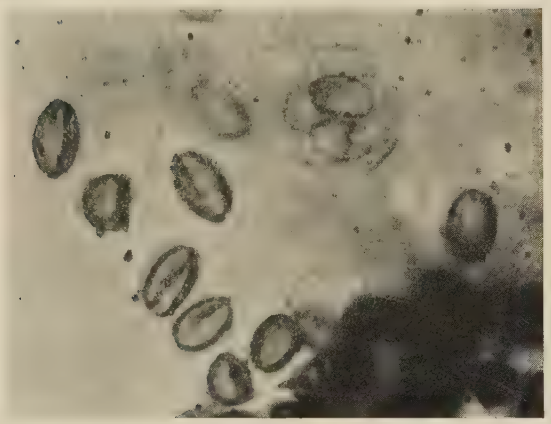

Fig. 3. Asco e ascospore (× 850 ca).

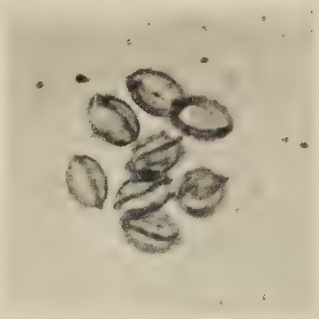

Fig. 4. Il contenuto di un asco (× 700 ca.).

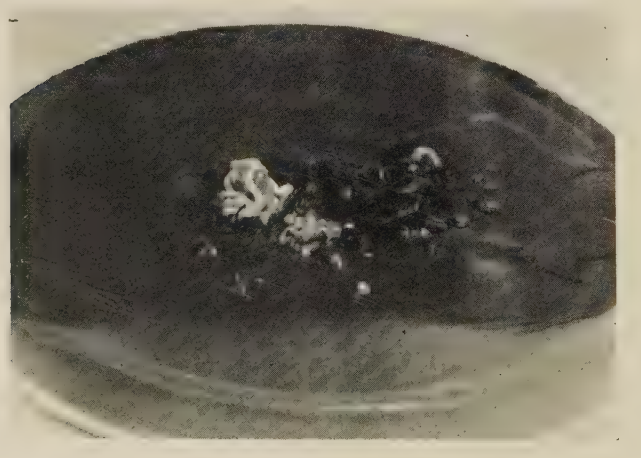

Fig. 5. Stromi di *Peyronellula mirabilis* su Czapeck agarizzato ($\frac{2}{3}$ delle dimensioni naturali).

tasselli di patata lo sviluppo è lentissimo e ridotto esclusivamente alla produzione di micelio roseo nel quale, anche in colture vecchie di 4 mesi, non ho visto formarsi neppure gli abbozzi dei periteci. Su paglia di segale (posta in tubi con un po' d'acqua e poi sterilizzata), il micelio è invece ridotto a tenui filamenti appena visibili, mentre si ha una ricca produzione di periteci. Su Czapeck agarizzato, lo sviluppo del fungo è estremamente stentato: dopo otto giorni un rado micelio roseo è appena visibile attorno all'inoculo, dopo un mese la colonia iniziale si è estesa pochissimo, ma in compenso il suo spessore e la sua compattezza rendono assai evidente il suo carattere di stroma, che si accentua maggiormente coll'invecchiare della coltura (Tav. XXXIII Fig. 5). L'esame di sezioni microscopiche di stromi dopo oltre 6 mesi di coltura, rivela la presenza in essi di numerosi periteci, in tutti gli stadi di maturazione, disseminati nello spessore dello stroma in modo analogo a quanto si osserva in natura.

Stentato e puramente miceliare è lo sviluppo del fungo sui substrati liquidi: brodo di carote, liquido di Czapeck. Alla superficie del liquido appaiono delle tenui isole di micelio d'un bianco appena tendente al roseo; queste coloniette galleggianti, costituite di ife di diametro leggermente maggiore (sui 3—4 μ) e più riccamente settate di quelle prodottesi sui terreni solidi, precipitano presto al fondo del recipiente, ove cessano di svilupparsi, mentre alla superficie se ne formano altre. In colture liquide vecchie di 6 mesi ancora non s'era iniziata la differenziazione dei periteci.

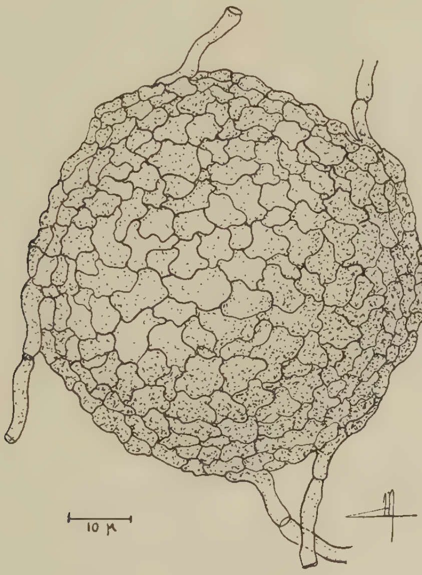

Fig. 6. Superficie di un giovane peritecio ottenuto in coltura (× 800).

Aspetti e dimensioni dei periteci, del loro eccipulo, degli aschi e delle ascospore ottenuti in coltura sono del tutto simili a quelli osservati in natura. Il fatto che in coltura i periteci si trovano facilmente isolati mi ha permesso di metterne in evidenza la superficie, che appare formata di pseudocellule a contorno ondulato, combacianti esattamente le une colle altre, a costituire un involucro continuo (Fig. 6).

La capacità di germinare delle ascospore ottenute in coltura sembrava dubbia, poiché quelle ricavate da colture vecchie di un mese non davano segno di germinare non solo in acqua piovana ed in acqua sterile, ma neanche in liquido di Czapeck ed in brodo di carote. Nell'ipotesi che la mancata germinazione fosse da attribuire o alla lentezza di rigonfiamento dell'involucro mucoso avvolgente le spore, oppure al non aver queste subito l'azione del freddo, cui sono soggette in natura durante l'ibernazione, od infine all'insufficiente stadio di maturazione delle ascospore ottenute nelle colture fino allora, ho provato: ad intaccare l'involucro mucoso con acidi; a far subire alle spore l'azione del freddo; ad impiegare colture più vecchie nelle quali, verosimilmente, le ascospore potevano aver raggiunto un più completo stadio di maturazione. Trattando le spore con acidi diluiti (solforico, cloridrico) e sciacquandole poi accuratamente in acqua sterile prima di saggiarne la germinabilità in liquido di Czapeck ed in brodo di carote, non ho avuto che risultati negativi. Ho invece registrato qualche risultato positivo tenendo una coltura, vecchia di un mese, sotto la neve per la durata di 15 giorni e per altri 20 giorni a 22°. Anche in questo caso, però, le ascospore che germinavano erano l'eccezione, le più rimanevano assolutamente inerti anche dopo 15 giorni di permanenza in preparati in goccia pendente tenuti a 22°.

Anche le ascospore di colture vecchie di tre mesi non germinavano che raramente. Dopo il periodo estivo ho ancora tentato di far germinare le ascospore prelevate da colture vecchie di 11 o 12 mesi ad ho avuto la gradita sorpresa di constatarne la facile germinabilità, in acqua sterile, segno che le ascospore necessitano, per giungere a maturazione, di un lungo periodo di quiescenza e, forse, sopratutto della permanenza in un ambiente molto povero di umidità.

Le esigenze del fungillo in fatto di temperatura nulla hanno di eccezionale: lo sviluppo, lento a 14°, trova il suo ottimo attorno ai 22°C. ed il suo massimo tra i 37 ed i 40°, ma già a 35° si riduce alla produzione di stromi fortemente rilevati, di aspetto mucido, di colore biancastro, nei quali soltanto con estrema lentezza vanno differenziandosi i periteci. Colture tenute per 5 giorni a 40° poi riportate a 22° non davano più segno di vita.

Particolare menzione merita invece l'adattabilità del fungo a terreni colturali a reazione molto varia. Il pH del terreno su cui il fungo si era sviluppato in natura risultò, in seguito a numerose prove (condotte col solito metodo della sospensione, centrifugazione e saggio colorimetrico), oscillare attorno a pH 7, ma lo sviluppo del fungo in terreno colturale non si rivelò affatto legato a tale valore del pH. Coltivando il fungillo su una serie di tubi di agar carote a pH crescente di 5 in 5 decimi, da 4,5 sino a pH 8, potei constatare che non soltanto lo sviluppo miceliare, ma anche la formazione dei periteci e la loro ulteriore differenziazione erano scarsamente influenzate dalle oscillazioni del pH e che neppure appariva evidente una tendenza del fungo a riportare i pH estremi ad un valore intermedio.

La forma conidica

La rapida formazione di piccole colonie disseminate su tutta la superficie dell'agar nutritizio, allorchè questo, preparato di fresco, è ancora coperto d'un velo liquido, faceva ritenere probabile, come già ho accennato, l'esistenza d'una forma conidica, analogamente, del resto, a quanto si verifica in natura. Tale forma conidica doveva però essere assai labile, poichè dopo 6—7 giorni cessava del tutto la produzione di nuove colonie, forse perchè venivano meno le condizioni ambientali (umidità) favorevoli; e d'altra parte l'esame microscopico, dopo tale termine, non permetteva più di trovare traccia di conidiofori o di conidi. Viceversa, seguendo lo sviluppo del fungo fin dai primi inizi ho potuto effettivamente sorprendere l'apparire, dopo poche ore di coltura, di una forma conidica (Fig. 7) identica a quella osservata sui giovani stromi raccolti in natura. Si tratta infatti di brevi conidiofori (30 — 50 × 3 — 3,5) jalini, semplici, poco distintamente settati, attenuati verso l'apice ove si producono, e rimangono per breve tempo agglomerati in capitoli di 10—15 μ di diametro, dei conidi jalini, oblunghi o cilindrici, arrotondati alle due estremità, misuranti 6 — 11 × 2,5 — 3 (per lo più 8 × 3), contenenti generalmente due guttule oleose. Tale forma conidica è però generalmente assai scarsa, tanto che non è sempre facile metterla in evidenza coll'esame microscopico. Per eliminare ogni dubbio circa la sua appartenenza al ciclo di sviluppo dell'ascomicete in istudio il metodo più ovvio sarebbe stato quello di ottenere delle colture monoconidiche, senonché la scarsità relativa dei conidi e la loro piccolezza rendevano l'impresa, non disponendo di un micromanipolatore, estremamente ardua; perciò girai la situazione nel modo seguente: avendo lavato con acqua sterile la superficie di colture di tre giorni appena, nelle quali era comparsa la forma conidica mentre i periteci non vi erano ancora neppure abbozzati, e distribuita la sospensione di conidi così ottenuta in numerosi tubi di agar-carote, in breve tempo comparvero in tutti le solite minute coloniette. Dopo tre giorni da questa comparsa, una metà dei tubi venne sottoposta a lavaggio con acqua sterile che fu distribuita in una seconda serie di tubi, che anch'essi produssero numerose colonie e dai quali si ottenne, collo stesso procedimento, una terza serie di colture. In tutti i tubi indistintamente, della prima, seconda e terza serie, comparvero sempre, dopo qualche tempo, i periteci.

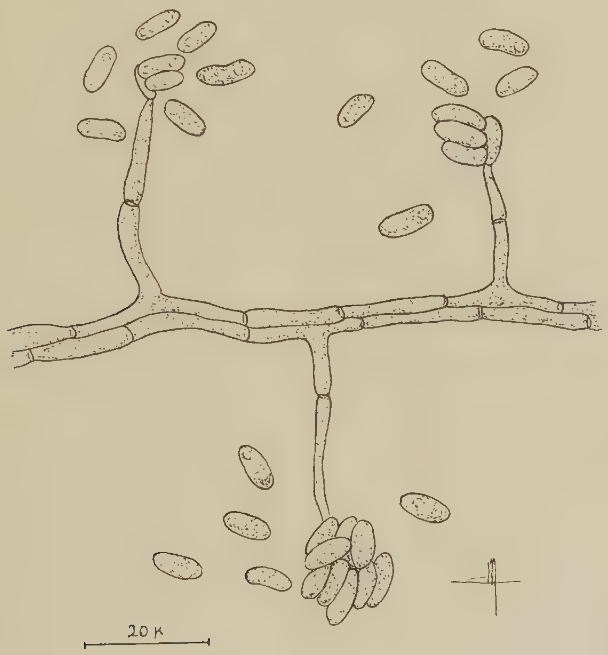

Fig. 7. La forma conidica: *Cephalosporium mirabilis* (× 800).

Molto più agevole fu l'ottenere colture monoascosporiche, le ascospore essendo di dimensioni relativamente notevoli e di colore scuro, quindi facili a distinguersi sopra un sottile strato di agar in capsule Petri, sul quale era stata distribuita con pipetta capillare una sospensione piuttosto diluita di esse, in modo da ottenere una disseminazione assai rada. Identificata sotto il microscopio, attraverso il fondo della capsula rovesciata, una ascospora bene isolata, si contrassegnava con un cerchio tracciato sul vetro, quindi, rimessa la capsula in posizione normale e sollevatone il coperchio, si asportava il pezzetto di agar delimitato dal cerchio e si inoculava in un tubo di coltura. Ho così ottenuto un certo numero di colture monosporiche le quali tutte hanno dato origine alla forma conidica sopra descritta: non c'è dubbio alcuno, quindi, che questa rientri nel ciclo biologico dell'ascomicete.

Merita tuttavia mettere in rilievo il fatto che queste culture monoascosporiche hanno rivelato l'esistenza di ceppi con diversa attitudine alla produzione di fruttificazioni conidiche, talune producendone in quantità relativamente cospicua, mentre altri — e sono i più numerosi — ne producono scarsamente.

Rapporti colla vegetazione

Ho già messo in evidenza il fatto che in natura gli stromi sono spesso inseriti su organi vegetali, sorta di tubi a largo diametro, avvizziti, di colore e consistenza del tutto dissimili dal sovrastante fungo e che ho identificati coi protonemi e rizoidi di un qualche musco umicolo. Nell'ipotesi che il fungo fosse un parassita — evidentemente facoltativo, data la facilità con cui lo si ottiene in coltura — del musco, l'ho seminato in capsule Petri su zolle di terreno su cui cresceva un musco prelevato nei dintorni di Torre Pellice. Naturalmente mi ero prima accertato che il fungo non preesistesse né sul musco né nel terreno. Sia che il musco non fosse del gruppo di quello nei cui protonemi avevo osservato il fungo (purtroppo l'assenza di qualsiasi forma riproduttiva non m'aveva permesso di tentarne la determinazione), sia che il fungo non abbia la capacità di attaccare protonemi e rizoidi vivi e sani, le prove furono negative.

Poteva presentare qualche interesse indagare se il fungo fosse suscettibile di esercitare qualche influenza sulla germinazione e lo sviluppo del frumento che sarebbe stato nuovamente coltivato nel terreno sul quale avevo osservato e raccolto il fungo stesso. Perciò delle cariossidi di frumento, di cui avevo controllato l'alto potere germinativo, furono sterilizzate esternamente con ,,uspulun'' al $2^0/_{00}$, accuratamente sciacquate in acqua sterile e deposte in tubi di coltura alcune su Czapeck agarizzato, altre su agar di carote acido, quindi a loro contatto fu inoculato il fungo. La germinazione delle cariossidi di frumento avvenne regolarmente, senza ritardo e senza anticipo rispetto ai controlli, ed il fungo stesso ebbe regolare sviluppo, per nulla influenzato dalla presenza delle piantine. Queste vennero dopo qualche tempo sottoposte ad accurato esame microscopico che però non rivelò mai in esse la presenza del fungillo. Si può concludere quindi che, almeno nell'ambiente così dissimile da quello naturale qual'è il tubo di coltura ed il substrato in esso contenuto, il fungo non esercita azione apprezzabile sulla germinazione e lo sviluppo del frumento.

Posizione sistematica

L'assegnazione del nostro fungillo ad un gruppo sistematico preciso di Ascomiceti non è facile, data la sua variabilità riguardo alla presenza o meno, allo sviluppo maggiore o minore ed alla forma di uno stroma ascocarpico. Infatti solo in natura ho osservato la curiosa forma di stromi a sfera cava, che poi si deprime nella sua parte superiore fino ad assumere l'aspetto di una ciotola emisferica, mentre in cultura lo stroma coincide con le singole colonie e si presenta generalmente sotto forma di placche rotondeggianti, ora poco ora molto rilevate a montagnola nella loro parte centrale e solcate da vallecole e corrugamenti radiali ora appena accennati ed ora (specie su taluni substrati, come l'agar-Czapeck) assai pronunciati. Ove il substrato scarseggia od è povero di elementi nutritizi, come nella parte superiore del ,,becco di clarino'' delle colture in tubo o sulla paglia di segale, lo stroma si assottiglia e si riduce fino, talora, a scomparire del tutto, i periteci formandosi allora in mezzo ad uno scarso e lasso intreccio miceliare che non può più considerarsi come stroma. Non sembra perciò, che si debba attribuire molta importanza, dal punto di vista sistematico, alla presenza o meno di cotesto stroma e neppure alla peculiare forma che esso presentava in natura, tanto più che non è escluso che essa fosse accidentale, non costante, e che in altre condizioni anche in pieno campo gli stromi possano mancare o assumere forma del tutto diversa.

Costanti sono invece la forma e la struttura degli ascocarpi, i quali possiedono un peridio (eccipulo) ben differenziato, sono del tutto astomi e quindi corrispondono a quel tipo di periteci che gli autori americani più recenti chiamano *cleistoteci*, contengono un numero indefinito di aschi rotondeggianti distribuiti senza alcun ordine e che li riempiono completamente. Molto importante è il fatto che la parete degli aschi si gelifica assai precocemente, lasciando libere le ascospore nella cavità periteciale. Importantissima e del tutto caratteristica è la forma delle ascospore.

In base ai caratteri enunciati, il fungo va senza esitazione ascritto, nella sottoclasse degli Euascomiceti (*Euascae* di Saccardo e Traverso), al raggruppamento, che si può definire *coorte*, dei Plectomiceti nel senso di GWYNNE—VAUGHAN, di WOLF e WOLF ed altri, funghi contraddistinti dall'avere ascocarpi astomi. Se, poi, prescindiamo dalla presenza, come abbiamo visto non costante, d'uno stroma, la disposizione disordinata degli aschi nell'interno dei periteci, che riempiono completamente, ci porta all'ordine delle Plectascali, od Euroziali o Aspergillali che dir si vogliano. Ci si arriva anche più direttamente col sistema del GÄUMANN (1949), il quale nella sottoclasse degli Euascomiceti contrappone le Plectascali (che egli, con poco rispetto delle regole di nomenclatura botanica, chiama *serie* (Reihe)) a tutti gli altri gruppi per aver aschi *precocemente* diffluenti (zerfallend).

Nell'ambito delle Plectascali (Euroziali), la famiglia che sembra più adatta ad accogliere il nostro fungo è quella delle Aspergillacee (o Euroziacee), che ne possiede tutte le caratteristiche essenziali, mentre tutte le altre famiglie (Gimnoascacee, Onigenacee, Elafomicetacee, Terfeziacee) sono da scartarsi per una ragione o per l'altra.

Però nell'ambito delle Aspergillacee nessuno dei generi finora descritti possiede le peculiari ascospore costate proprie del nostro fungo, e neppure, del resto, nell'ambito delle altre famiglie. E' questo un carattere tanto importante, da giustificare la creazione d'un nuovo genere per il quale propongo il nome di *Peyronellula*.

In quanto alla forma conidica, essa trova il suo posto nel genere *Cephalosporium*, di cui costituisce una specie nuova, poiché le sue caratteristiche non coincidono con quelle di nessuna delle specie finora note.

Dò ora la diagnosi del nuovo genere e della nuova specie:

Peyronellula novum genus

(eximio mycologo Beniamino Peyronel dicatum)

Perithecia (cleistothecia) globosa, astoma, excipulo membranaceo perfecte evoluto, pseudoparenchymatico praedita, in stromatibus plus minusve evolutis rite immersa. Asci globosi, perithecia inordinate complentes, octospori, mox diffluentes; ascosporae (sporidia) ellipsoideae, continuae, primum hyalinae et strato mucoso vestitae, deinde fuscae et costis longitudinalibus ornatae.

Peyronellula mirabilis n.sp. — Perithecia (cleistothecia) globosa, astoma, minuta, 80—120 μ diam., excipulo pseudoparenchymatico hyalino 10—20 μ crasso donata, plerumque in stromatibus plus minusve evolutis immersa et densissime constipata, rarius libera vel in mycelio laxo conglobata; asci globosi, 20—24 μ diam., octospori, mox diffluentes; sporidia ellipsoidea, continua, immatura hyalina, levia et strato mucoso crasso obvoluta, matura pelliculā fuscidulā in 3—6 (plerumque 4—5) costis longitudinalibus corrugatā, ex muco exsiccato et collapso formatā, vestita, 10 — 14 × 5 — 6,5, plerumque 11 × 6. Stromata *in natura* terricola, in superficie humi gregaria, albida vel pallide rosea, typice primum sphaeroidea, cava, deinde parte superiori collabescentia formamque cyathi sumentia, inferne per radicem brevem matrici infixa, 300—500 μ lata; cavitas stromatorum mycelium gossypinum laxum continet, paries contra constat praecipue ex peritheciis copiosissimis in hyphenchymate parco sed arcte contexto, extus pseudoparenchymatico inclusis. *In cultura,* in substratis solidis, stromata monticuliformia ambitu plus minusve rotundo vel irregulari, valleculis radialibus saepe sulcata, compacta, ex hyphys crassiusculis, ramosis, septatis, hyalinis, plasmate oleoso repletis constituta. Status conidicus: *Cephalosporium mirabilis* n. sp. — Conidiophora simplicia, sursum leniter attenuata, continua vel pauciseptata, hyalina, 30 — 50 × 3, saepe in flocculis erectis vel assurgentibus, albidi vel pallide roseis, ex hyphys parallele decurrentibus constantibus inserta, apice capitula conidiorum globulosa, 10—15 μ lata gerentia; conidia hyalina vel in cumulo dilute rosea, oblongo-ellipsoidea vel subcylindrica, uni-biguttulata, 6—11 × 2,5—3, plerumque 8 × 3, muco pauco conglutinata.

Habitat: ad terram humosam agri triticini et in cellulis hypogaeis protonematum emortuorum Muscinearum, prope Pinarolium (Pinerolo) Pedemontii, Ital. sept.

Riassunto

Viene descritto un nuovo genere di Plectomiceti al quale viene dato il nome di *Peyronellula* dedicandolo al micologo Beniamino Peyronel.

Il nuovo genere presenta dei cleistoteci a parete ben definita, completamente riempiti di un numero indefinito di aschi rotondeggianti, distribuiti senz'alcun ordine. Gli aschi, a parete diffluente, contengono otto ascospore ellittiche, continue, a maturità brune e caratteristiche per essere munite di costole longitudinali.

La specie in base alla quale è descritto il nuovo genere, denominata *Peyronellula mirabilis,* venne rinvenuta alla superficie del terreno in un vigneto sito nei dintorni di Pinerolo (Piemonte, Italia). Di essa è stato ottenuto l'isolamento in coltura pura e si è individuata la forma conidica appartenente al genere *Cephalosporium* di cui costituisce una nuova specie, denominata *Ceph. mirabilis.*

Del genere e delle specie nuove è data una minuta descrizione corredata dalle relative diagnosi latine.

Résumé

L'auteur décrit un genre nouveau de Plectomycètes qu'il nomme *Peyronellula* en le dédiant au mycologue Beniamino Peyronel.

Le nouveau genre présente des cléistothèces globuleux, pourvus d'un péridium bien défini, fermé, complètement rempli d' asques sphériques en nombre indéterminé et amassés sans ordre apparent. Les asques, dont la paroi se gélifie à maturité, contiennent huit ascospores elliptiques, non cloisonnées, qui en mûrissant deviennent brunes et ornementées de crêtes longitudinales qui leur donnent un aspect très caractéristique.

L'espèce sur laquelle se base ce nouveau genre, *Peyronellula mirabilis,* fu trouvée à la surface du sol d'un vignoble aux environs de Pignerol (Piémont, Italie). La forme conidienne qui a été, ainsi que la forme parfaite, obtenue en culture pure, appartient au genre *Cephalosporium,* dont elle constitue une nouvelle espèce, nommée *Cephalosporium mirabilis.*

La diagnose latine complète la déscription du nouveau genre et des nouvelles espèces.

Summary

Peyronellula, a new genus of Plectomycetes dedicated to the mycologist Beniamino Peyronel, is described.

The spherical, astomous cleistothecia of the new genus have a well defined peridium and are full of an indetermined number of spherical asci. The asci, who disintegrate at maturity, contain eight elliptical, continuous, dark ascospores, fully characterized by longitudinal crests.

The type species of the new genus, named *Peyronellula mirabilis*, was found on the soil of a vineyard near Pinerolo (Piedmont, Italy).

A conidial stage of genus *Cephalosporium* has been also found to which the name *Cephalosporium mirabilis* is given. Both ascophorus and conidial stages were grown in pure culture.

Full accounts of the genus and the new species, with thoroughly latin diagnosis, are given.

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Some Aspects of the Pathogenicity of Candida albicans in Laboratory Animals

by

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Only a few papers dealing with the pathogenicity of the so called medical monilias have been published.

REDAELLI (3) injected rabbits, guinea pigs and dogs by intravenous, intra-arterial, subcutaneous, intraperitoneal, intracorneal and intrapleural routes.

MACKINNON and RODRIGUEZ GARCIA (2) used the intravenous route in the rabbit, intracardiac injections in the guinea pig and several other routes in the remaining animals.

BENHAM (1) describes the skin and organ lesions, SALAZAR LEYTE (4) eossinophilic tissue response and hemoptysis in bronchopulmonary moniliasis. STOWALL and PESSIN (5) gives a clinical description in rabbits similar to those commonly found in any toxic state.

While studying the pathogenicity of some 30 strains of *C. albicans* (from vaginal moniliasis) in rabbits, it was found that most animals developed nervous symptoms such as paresis, stiffness of the neck muscles etc.

These findings prompted us to study whether other laboratory animals would show similar symptoms after inoculated with *C. albicans*.

In the present paper we describe hitherto unreported symptomatology and pathology in laboratory animals after intravenous inocculation with *C. albicans*. Additional information on the comparative susceptibility of the rabbit, rat, mouse and guinea pig to infection with *C. albicans* is also given.

Material and methods

A suspension of our *C. albicans* M-28 grown for 48 hours on Sabouraud's dextrose agar was made in saline and counted in the haemocytometer.

20 rabbits, 16 guinea pigs, 12 rats and 18 mice were inoculated intravenously with 1 million cells per hundred grams of weight each.

Each group was divided in two according to sex. A clinical examination was made, and after death the organs were fixed in 10 % formaldehyde and sections were stained with Gram stain and hematoxylin eosin.

When possible, autopsies were carried out immediately after death, otherwise the material was kept in the refrigerator at 0° C. overnight.

Results

The clinical symptoms displayed by the inoculated animals are to be found in Table I.

TABLE I. *Symptoms.*

	Rats	Mice	Guinea pigs	Rabbits
Sleepiness	50 %	17 %		83 %
Nasal Hemorrhage	83 %			
Ocular Hemorrhage	83 %			
Stiffness of neck	17 %			33 %
Paresias	67 %	17 %	50 %	
Choreatic movements	17 %			

Some rabbits showed lateral pendular movements of the head and nystagmus. All reflexes were hyperactive. (Fig. 1).

Those animals having involvement of the dorsal and lateral muscles of the neck walked in a circular way. Those in which paresis developed in the legs fell to the side involved. Attempts at standing resulting in rolling over.

Excitation provoked convulsions which subsided shortly, the animal remaining inert on the floor of the cage.

Only rats showed nasal hemorrhage. These hemorrhages appeared after a few days, staining the hair of the nostrils. (Fig. 2) Some rats showed a rim of blood around the eyes mainly on the medical rim of the orbit. A careful observation of the conjunctival blood vessels did not show pathologic alterations so the blood might have come from the nose.

The average of survival of the different animals can be seen in Table II.

TABLE II. *Average survival after injections.*

	Days	Percentage of death
Rats	5	100
Mice	15	100
Rabbits	5	95
Guinea Pigs	11	83

Post mortem examinations of the urine taken by bladder puncture showed great numbers of leukocytes, casts of different types, renal cells, blastospores and some hyphae.

At autopsy the organism was found mainly in the kidney, (Fig. 3) brain and gall bladder. Positive cultures were also obtained from prostate, seminal vesicles, and the eyeball, and there were instances of finding the organism in almost all organs.

The pathology described below is concerned mainly with the lesions found in the brain, vagina, internal genitalia, and nose of the animals.

Other organs were not mentioned since their description has already appeared in the literature (3,4). By far, the most impressive lesions were found in the brain.

Brain abscesses were seen in the gray and white matter. They were conspicuous in the rabbit. In this animal the number varied from scant to relatively numerous. Size ranged from pin point to about 1 mm. in diameter, protruding slightly from the surface.

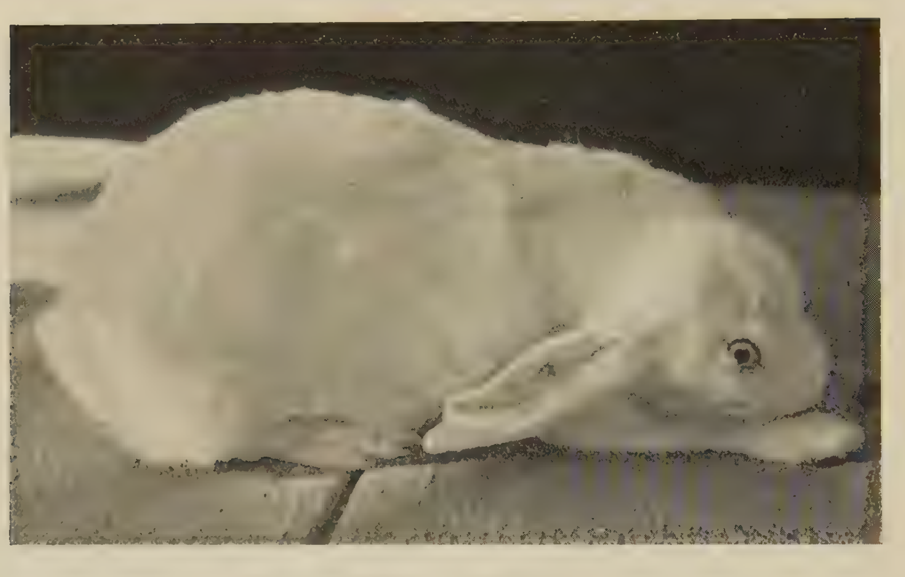

Fig. 1. Choreatic movements rabbit.

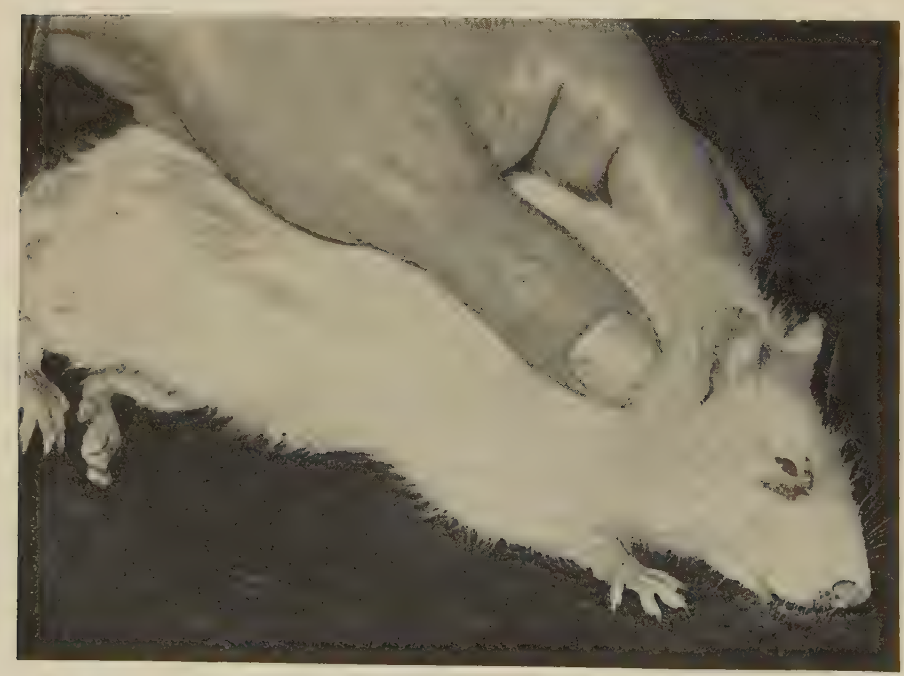

Fig. 2. Perinasal and periocular hemorrhage rat.

TABLE III. *Brain pathology.*

Species	No. of brains examined	Encephalitis	Severe
Rats	9	9 100%	5 55%
Mice	34	13 54%	7 29%
Rabbits	20	18 90%	14 77%
Guinea Pigs	16	16 100%	12 75%

Those in rats were not as numerous and were pin point in size. In guinea pigs they were not clear cut and in mice they were rare.

The table shows that rabbits and guinea pigs presented brain involvement with far greater regularity than rats, and at the same time the lesions observed were much more extensive and numerous than in rats.

Three lesions could be observed in different animals. Combinations of the three forms were rather common.

1. Mononuclear encephalitis in the form of perivascular infiltrates; frequently, especially in rats this was the only manifestation of inflammation. Often a few scattered lymphocytes were seen around the capillaries. In other instances, the Virchow-Robin spaces were dilated apparently by edematous fluid and filled with round cells which spread from larger vessels along the branches and finally around the capillaries. Tangential sections through such pericapillary infiltrates impressed us as being small focal encephalitic foci. The relation to the vessel could always be traced back. In this form of brain involvement we did not observe yeast cells in the infiltrate, even if the Virchow-Robin spaces were heavily infiltrated.

In a more advanced stage the process seems to be intense vascular congestion, perivascular proliferation of the glia and desquamation of the vascular endothelium. Outside the abscesses some pseudohyphae were observed also in the lumen of capillaries.

2. Abscess formation was fairly common, more frequently in the larger rodents than in rats. Either scattered single foci or large confluent lesions were observed. The isolated lesion was variable in size generally round, sharply circumscribed, the center necrotic and invaded by polymorphonuclear leukocytes, the periphery formed by a mixture of polymorphonuclear leukocytes and round cells. There were also eosinophiles (4). Frequently a marked hyperemia existed but not so much around small isolated foci as in the generalized form. The hyperemia included mainly the meninges even in cases where no inflammatory reaction was found in the pia-arachnoid.

In about 20 % of the cases abscesses lying immediately below the pia broke through and filled the subarachnoid space locally. Surprisingly enough, such meningitides generally remained local and if a spread was observed, round cells and not polymorphonuclear leukocytes prevailed a short distance from the ruptured abscess.

In several cases abscesses were confluent and covered a large part of the examined slides. In such confluent cases, the differentiation into a central necrotic area and a round celled periphery was much less outspoken. Repeatedly large areas of necrosis and polimorphonuclear proliferation were seen. Sometimes vessels lying in such areas were necrotic but no thrombotic phenomena were ever observed.

This type of lesion always showed yeast cells and pseudohyphae even if the number frequently seemed to be in contrast with the severity of the inflammatory response.

It was more frequent than not to find abscesses in combination with perivascular infiltrates as described under 1.

3. Granulomatous lesions, especially in larger animals, appeared to be expressions of a subacute to chronic response to the presence of the organism. Sometimes we observed small circumscribed, always rounded, nodular lesions composed of elongated clear cells which, if not identical with, at least had the morphologic characteristics of epitheloid cells.

In other instances granulomas composed of epitheloid cells, a peripheral round cell infiltrate and occasional giant cells of the Langhans type were numerous and scattered through white and gray matter. If such foci touched the meninges local round cell meningitis occurred but the inflammation was always restricted to a rather small area.

The number of yeast cells in the granulomatà seemed to be less than in the abscess formation, but certainly the difference was not great enough to be conclusive.

In the fluid of the lateral ventricles, a great number of lymphocytes and desquamated cells were suspended. There were also scarce polynuclear neutrophiles, red blood cells and an occasional macrophage. The folds of the choroidal plexus were very thickened by edema and there was marked infiltration of mononuclear cells. In some zones, the lining epithelium was gone. In the plexus scarce blastospores together with some pseudo hyphae were seen.

Vaginal bleeding was not demonstrated to be due to inflamation caused by local yeast deposits. If capillary damage by toxic products existed, it was not visible in our sections.

Some of the rats showed hyperemia of the nasal vessels.

Uterus and ovary were remarkably free of any inflammatory response; several pregnancies actually developed apparently normally,

In the male rats the prostate was 4 times the site of granulomatous and abscess lesions in a series of 7. It was found enlarged, two to three times. Some specimens showed both lobes so filled with abscesses that there was hardly any normal tissue left, the appearance being similar to that observed in some rabbit kidneys. On section, the acini were filled with pus. A great number of blastospores and filaments were found. It is striking that great numbers of the filaments located in the acini did not take the Gram stain, appearing hyaline. The interstitial tissue showed a marked inflammatory reaction. This prostatic involvement was reminiscent of other generalized processes that have frequent prostatic localization, i.e. generalized blastomycosis.

In one guinea pig we saw abscesses in the testicle but the Gram stain did not disclose yeast elements. Therefore non-specific orchitis is not ruled out.

In 3 out of 7 guinea pigs abscesses were observed in the fat body surrounding the epididymis.

In the other animals (mice, rats, rabbits, guinea pigs) no inflammation was seen of any of the genitalia even though examination of the testicle, epididymis, prostate and seminal vesicle in males, ovaries, oviducts and uterus in females was done routinely.

Comment

Redaelli (3) states that the parasite is introduced into circulation as an embolus and as such it stays in the small vessels with obstruction of the lumen. In the middle-size vessels they would form a little thrombus in which the

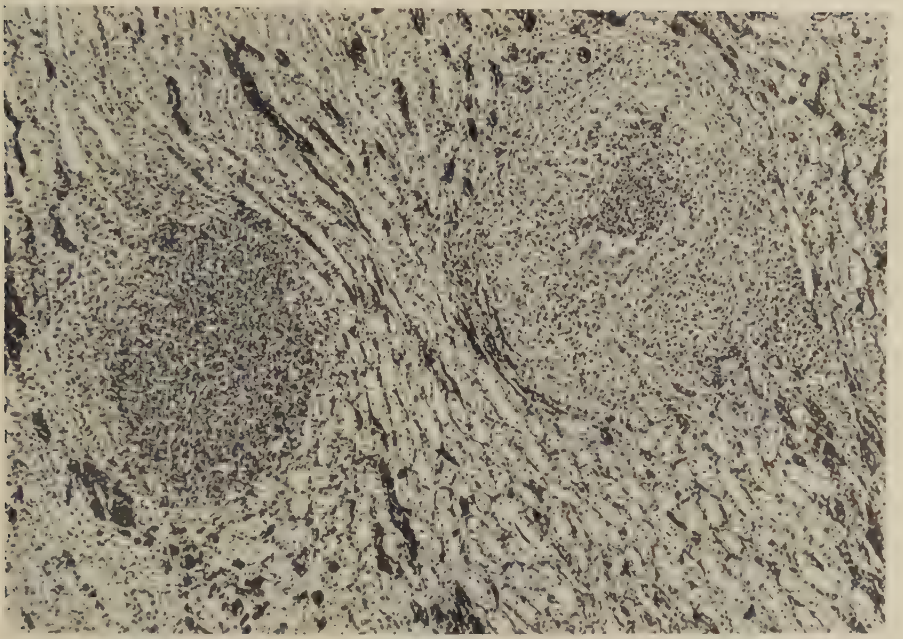

Fig. 3. Granulomatous and exudative (abscess-like) lesions in the same organ (kidney) rabbit.

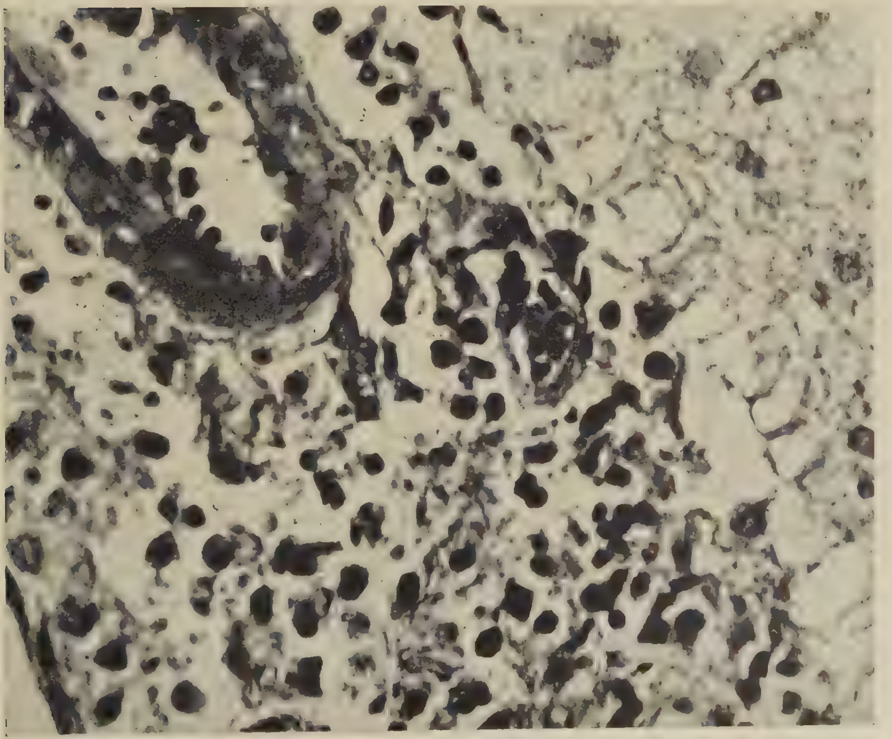

Fig. 4. Mononuclear meningitis; vicinity of encephalitic focus. rabbit.

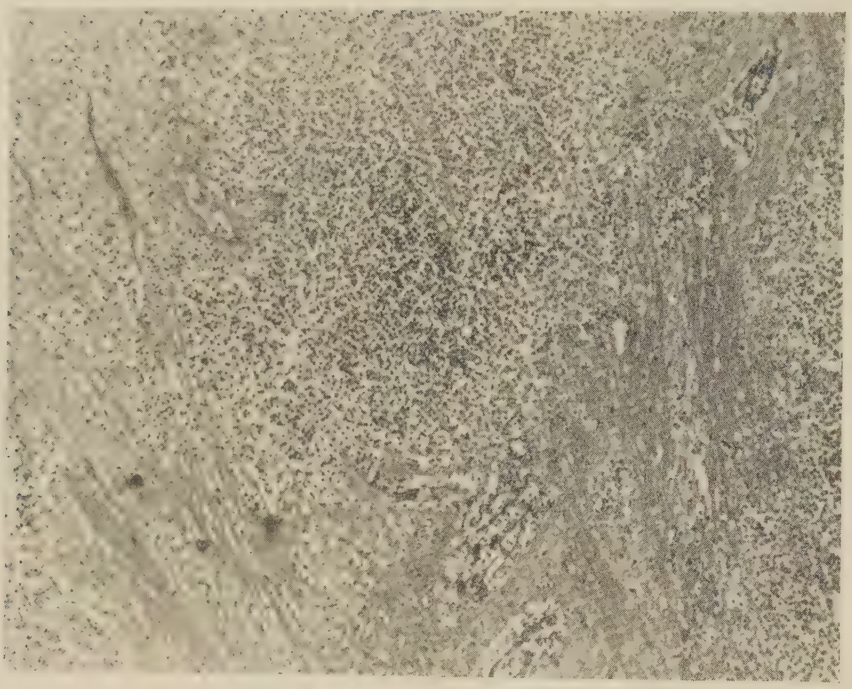

Fig. 5. Widespread and confluent encephalitis rat.

fungus can develop and penetrate through the walls of the vessels into the surrounding tissue. SALAZAR LEYTE (4) described similar findings.

On the other hand, STOVALL and PESSIN (5) injecting intravenously killed blastospores of different sizes of *C. albicans*, *C. parakrusei* and *C. tropicalis* could not produce any type of gross or microscopic lesion.

In our sections we have seen either blastospores or pseudohyphae in the lumen of capillaries occasionally surrounded by desquamated endothelium Outside the capillaries red cells were seen evidently extruding by diapedesis through defects in the endothelial layer.

This fact suggests the possibility of toxic damage produced locally by yeast cells.

Summary

A study of nervous and hemorrhagic signs of experimental moniliasis and data on the comparative susceptibility of laboratory animals to *C. albicans* are presented.

Paresis, ataxia, choreatic movements, unilateral neck muscle contractures, etc. were observed in every animal species. Hemorrhagic signs were also common and consisted of epistaxis and bloody eyes (only in rats).

The susceptibility to *C. albicans* is in the mentioned order most outspoken in the rat, than the rabbit, guinea pig and mouse.

A special selectivity for prostatic localization was observed in rats, but was absent in the other species.

The possibility of toxic endothelial damage is mentioned.

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Studies of the Dimorphism Mechanism in *Saccharomyces cerevisiae* [1] [2]

by

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Introduction

The pathogenic fungi may be divided into two groups. Those which can invade only the superficial skin are called "dermatophytes"; those which can infect not only the skin but also the deeper organs usually belong to the group of yeastlike fungi. There are a number of yeastlike pathogenic fungi which occur in a yeast (Y) phase *in vivo* and under special conditions *in vitro*, but which usually grow in a mycelial (M) phase *in vitro* when incubated at room temperature. This characteristic has been termed "dimorphism". The dermatophytes do not show dimorphism.

Since the dermatophytes exhibit mycelial growth and grow well *in vitro* at temperatures from 25° C to 38° C (NICKERSON and WILLIAMS, 1947) it is possible that their inability to invade the deeper tissues may be correlated with the absence of an intracellular mechanism responsible for yeast to filament (F) (Y → F) conversion in the presence of bodily conditions that will suppress mycelial growth. Thus attempts by SULZBERGER (1929) and BROCQ-ROUSSEU et al. (1926) to precipitate a systemic and deep infection with dermatophytes that do not exhibit dimorphism failed. Inoculations by these workers of large doses of spores of *Trichophyton* and *Microsporum* into guinea pigs did not cause an infection in any of the internal organs.

BAKER, MRAK, and SMITH (1943) have suggested that if the parasitic condition is considerd as abnormal for the devlopment of the mycelial form of the fungus, possibly the development of the fungus has been reduced to

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[1] A portion of this work has been presented before the Genetics Society of America meeting in Columbus, Ohio, Sept. 11, 1950 (SCHERR, 1950).

[2] This report is taken from a dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Kentucky, Lexington.

The author wishes to express his appreciation to Drs. R. H. WEAVER, M. SCHERAGO, MARGARET HOTCHKISS, H. P. RILEY, and W. D. VALLEAU, all of the University of Kentucky, for their ever-present interest in connection with this work.

a form which produces the greatest number of reproductive structures with the least synthesis of new protoplasm. In other words, the environmental conditions imposed by animal tissues restrict mycelial development, which is less profitable from a reproductive standpoint. It should logically follow from this that any agent which would tend to transform the established yeast phase *in vivo* to the mycelial phase might serve to arrest the multiplication of the pathogen. This, presumably, would occur because of a metabolic blocking action within the parasitic cell, resulting from an enhancement of mycelial formation by the introduced agent on the one hand and a tendency to maintain the yeast phase by the bodily mechanisms on the other.

Because of the postulated possibility of treating infections due to yeastlike pathogenic fungi with Y → F enhancing substances, it was felt that a more extensive investigation of the effects of various compounds in inducing Y → F formation *in vitro* might be profitable. *S. cerevisiae* was chosen for this investigation for the following reasons:

1. It could be handled more easily because it is non-pathogenic.
2. Cause and effect of Y → F formation could be more easily correlated because it does not normally form a filamentous phase.
3. It was felt that, because *S. cerevisiae* is closely related antigenically and phylogenetically to a number of the yeastlike pathogenic fungi, many observations made using the former might be readily applicable to the latter.

Hansen (1886) showed that *Saccharomyces* strains incubated at a temperature of approximately 20° C grow in characteristic round, budding cells, while a lowered temperature of incubation gives rise to growth in the form of filaments. Even before Hansen, as Levan (1947) points out, brewers had observed such so-called involution forms (filaments) in aging yeast cultures. The sketch of a yeast from an old wine film reproduced by Lafar (1911) from Aderhold (1894) shows the identical type of filament formation later described by many other workers. Lepeschkin (1903) observed the formation of a mycelium by *Schizosaccharomyces Pombe* and *Schizosaccharomyces mellacei*. The mycelial stage remained constant during numerous generations and this worker was unable to secure reversion to the original yeast phase. Lepeschkin's drawings of these filamenents are nearly identical with those of Aderhold (1894).

Anderson (1917) reported a decided change in the form of yeast cells in old cultures. In many cases the cells had elongated while remaining attached to each other, forming a hyphal thread constricted at the ends of the cells. In these old cultures giant cells, frequently ten times the diameter of the normal cells, were also present.

Zikes' (1920) studies on the effect of temperature on yeast morphology indicated that lower temperatures give rise to elongated cells in different yeast genera and that there is also a tendency at lower temperatures for yeast cells to hang together in colonies. He found, in addition, that the normal appearance of the yeast cells was restored after incubation at an optimum temperature. Zikes termed these aberrent forms "fluchtige Varietaten", or unstable variants. Richards (1928) observed that cultures of *Saccharomyces cerevisiae* maintained within the narrow temperature limits of 29.5°—30.5° C exhibited the maximum deviation from normal cell shape (enhancement of Y → M) when compared to cultures incubated at either side of this temperature range.

Early work on the occurrence of spantaneous and induced variations in yeasts showed that some of the more commonly occurring variants developed as mycelial races from normal unicellular ones. Nadson and Krassilnikov (1932) reported the occurrence of spontaneous variants in a yeastlike fungus, *Guilliermondella selenspora.* Five races emerged, of which one was characterized by abundant mycelium, one was entirely yeastlike and formed no mycelium, and the other three showed somewhat intermediate characters. PUNKARI and HENRICI (1933) described the occurrence in *Torulopsis pulcherrima* of spontaneous variations in color (red and white) and texture (smooth and rough). Roughness was correlated with the development of a mycelium in both the red and white variants. ROCHLINA (1934, 1936) obtained variants of *S. cerevisiae* by using radium. Among these was a stable race composed of cells with numerous projections which were considered to be undeveloped buds with thin walls, but they might well have been sprouting hyphae from yeast cells.

MICKLE and JONES (1940) reported that dissociation from smooth to rough colonies of *Candida albicans* could be accomplished using 0.25 % lithium chloride or 3 % immune rabbit serum. The early growth of smooth forms on Sabouraud's agar showed round or slightly oval budding cells. Rough colonies were composed of elongated cells, some having bizarre shapes, and many of these approached the appearance of mycelial filaments.

NADSON (1937) summarized a common pattern of morphological variation in yeasts, indicating that this pattern is found whether the variations are spontaneous or induced. He stated that there is a tendency for the normally globular yeast with smooth colonies to give rise to forms which are cylindrical or sausage-shaped, then to a pseudomycelium, and finally to a true mycelium; these trends are accompanied by an increasing roughness of the colonies.

SEGAL (1938, 1939) described abnormal forms of yeasts resulting from their exposure to fusel oil and higher alcohols. Abnormal budding resulted, giving rise to cell chains, but these abnormal forms disappeared when the yeasts were subcultured in ordinary nutrient media. Yeasts exposed to the same alcohols in water produced no unusual forms, indicating that the cells were affected only while in the process of multiplication.

A fuller discussion of and numerous other references relative to this topic will be found in HENRICI's (1941) review.

A number of studies have been reported on the effect of carcinogenic chemicals on yeast cells. Giant cells and increased morphological differentiation of cells within the colony of *Saccharomyces ellipsoideus* Hansen were produced when the cultures were subjected to a solution of methylcholanthrene at a concentration of 1/50 of saturation in distilled water (DODGE and DODGE, 1937). DODGE (1940) found that cultures of *S. ellipsoideus* subjected to methylcholanthrene yielded occasional giant cells and more frequent division. DODGE, DODGE, and JOHNSON (1941), however, found only small differences in the dry weights of cultures that had been grown under standard conditions and those that had been exposed to methylcholanthrene. COOK, HART, and JOLY (1939) showed that the effect of 1 : 2 : 5 : 6-dibenzanthracene on the proliferation and respiration of *S. cerevisiae* can be stimulative or inhibitory, depending upon the concentration of the carcinogen.

ROBINSON and STIER (1941) assayed, using the coleoptile test, the amount of auxin in yeast cultures grown in various media and found that with a high auxin concentration in the medium many of the yeast cells occurred in the form of filaments. The suggestion was made by these workers that the increased concentration of auxin might have been responsible for the F forms of the yeast.

BAUCH (1941), using camphor, was the first person to experimentally produce a high incidence of the filamentous forms in yeast. Later, BAUCH (1942) found greatly enlarged cell mutants in yeast cultures which were exposed to 3,4-benzpyrene and methylcholanthrene; 1 : 2 : 5 : 6-dibenzanthracene was without effect.

The investigations of LEVAN and SANDWALL (1943) and LEVAN (1944, 1947) established that the camphor (cf-) reaction was not by any means limited to camphor, since most of 50 organic compounds, many of which had narcotic properties, elicited the camphor reaction in yeast cells. Of the compounds examined that demonstrated camphor activity, 18 were aliphatic, 6 were alicyclic, 14 were aromatic, and 2 were heterocyclic. The few bi -and polycyclic aromatic substances tested showed no activity, with the exception of alpha-naphthalene acetic acid, which "showed certain tendencies to inducing the camphor reaction". The most notable camphor effect was caused by camphor, for which the percentage occurrence of cf-forms increases in solutions up to 0.007 molar, in which concentration almost all living cells are such forms.

The outstanding features of the camphor reaction were:

1. The cells tended to hang together in colonies.
2. The reaction was incomplete, since normal cells were always found intermingled with cf-forms, even at the highest concentrations tested which would still permit cellular growth.
3. The morphology of the affected cells showed consistency only in that cell elongation and the inhibition of cellular division were pre-eminent. Aside from this, cells affected by different chemicals showed irregularly swollen cell aggregates, long filamentous cells, and numerous grotesque-looking cells of varied sizes and shapes. Note fig. 1 and 2, which are reproduced from LEVAN's (1947) paper.
4. There was an almost consistent inverse relationship between the cf-activity of the chemicals tested and their water solubility.
5. The cf-reaction induced by different chemicals was further enhanced by low temperatures (9°—20° C) and impeded by higher temperatures (30° C). Occasionally it was observed that low temperatures alone would favor cf-growth.
6. The cf-reaction was reversible in that cf-cells would begin to show normal budding processes if the concentrations of the cf-agents began to fall below their threshold value for cf-activity or if the yeast cells acquired a resistance to the cf-chemicals.

SUBRAMANIAM (1945) exposed cells of *S. cerevisiae* growing in wort medium to acenaphthene for 6 hours and found that "long threadlike mycelial growths" (fig. 3) became very common.

NICKERSON and VAN RIJ (1949) were able to shift the Y → M equilibrium toward the Y phase using organisms which very readily formed a mycelium. They found that a concentration of 10^{-2} molar of cysteine inhibited mycelial formation in cultures of *Candida albicans, Candida tropicalis, Trichosporon capitatum,* and *Hansenula anomala.* That this phenomenon could not be explained solely by redox potential differences was demonstrated by exposing these cultures to a 10^{-2} molar concentration of ascorbic acid, with no resultant inhibition of Y → M. It also appeared that some specificity was required of the — SH donor, since sodium thioglycollate was not as effective as glutathione, which in turn was less effective than cysteine, in inhibiting Y → M.

NICKERSON and VAN RIJ (1949) also showed that the characteristic effect of cysteine on *C. albicans* was to produce very short hyphal cells with very close spacing of blastospore clusters. A 10^{-2} molar concentration of cysteine reversed the enhancement of Y → M exhibited by subfungistatic concentrations of penicillin and cobalt ions. The Y → M promoting effects of cobalt were also antagonized by oxine (8-hydroxy quinaline). An examination was made of other metal ions by these workers, but $CuSO_4$, $MnCl_2$, $NaHAsO_3$, and Na_3AsO_4 were without effect on Y → M formation. Only boron ($K_2B_4O_7$) showed a significant effect, but to a much smaller extent than that shown by cobalt. Cells of *S. cerevisiae* exposed to penicillin became more elongated and grew in the form of filaments. Again, cysteine antagonized the effect of the penicillin.

The work of these authors supports their contention that "*cell division, as a process distinct from growth,* depends upon the maintenance of functional intracellular sulfhydryl groups".

Materials and methods

The culture of *S. cerevisiae* used in this study was originally isolated from a commercial pressed yeast cake by Dr. MARY HARDIN of the Department of Bacteriology, University of Kentucky, and has been carried as a stock strain in that department. Its identification was confirmed in this study by culturing in a synthetic medium (# 20 medium) containing different compounds as sole sources of carbon. Raffinose, sucrose, maltose, d-galactose, and glucose were utilized as sole sources of carbon; lactose, inulin, dulcitol, d-mannitol, d-sorbitol, d-xylose, d-arabinose, cellobiose, and melibiose were not utilized. Urea was utilized as a sole source of nitrogen. This is in accord with the STELLING-DEKKER (1931) scheme of classification.

The synthetic medium referred to as # 20 medium is essentially a modification of WICKERHAM'S (1946) medium.

One liter of the finished medium contains:

A.	1) KH_2PO_4	1 g
	2) $(NH_4)_2SO_4$	5 g
	3) $MgSO_4 . 7H_2O$	0.5 g
	4) NaCl	0.1 g
	5) $CaCl_2 . 2H_2O$	0.1 g
	6) Biotin	20 μg
	7) Inositol	2000 μg
	8) para-Aminobenzoic acid	200 μg
B.	9) Vitamin A	167.0 U.S.P. units
	10) Vitamin D	33.4 U.S.P. units
	11) Vitamin C (ascorbic acid)	1.67 μg
	12) Vitamin B_1 (Thiamine HCl)	11.1 U.S.P. units
	13) Vitamin B_2 (Riboflavin)	13.3 μg
	14) Vitamin B_6 (Pyridoxine HCl)	3.3 μg
	15) Niacinamide	166.8 μg
	16) Pantothenate acid	66.7 μg
	17) Carbon source	0.1 molar concentration
	18) Tap water to volume	

The salts indicated in group A were made up in a stock solution at twice the desired final concentration. Stock solutions of biotin, inositol, and para-aminobenzoic acid contained 5 μg/ml, 1 mg/ml, and 0.1 mg/ml, respectively. The ingredients in group B compose the commercial vitamin preparation known as "Vi-Syneral", manufactured by the U.S. Vitamin Corp. One ml of the Vi-Syneral was diluted 1 : 25 with distilled water to yield a stock solution. All solutions were stored in a refrigerator when not in use.

To prepare, for example, 500 ml of this medium, 250 ml of stock solution A, and 2ml of botion, 1 ml of inositol, 1 ml of para-aminobenzoic acid, and

4 ml of Vi-Syneral stock solutions were added to a 500 ml volumetric flask. The amount of carbon source added, depending on the compound used, was such as to yield a final concentration of 0.1 molar. Tap water was added to the mark and the completed medium was sterilized by filtration through a Seitz filter.

Although WICKERHAM (1946) included the trace elements boron, copper, iodine, iron (ferric), zinc, manganese, and molybdenum in his medium, he indicated that the addition of trace elements appeared to have little influence on growth even when a number of fastideous yeasts were tested. He concluded that sufficient trace elementes are present as impurities in other ingredients to supply the necessary ions.

All tap water referred to in this report was Lexington city water. An analysis of this water, made during Sept. 1948, was kindly provided by Mr. E. E. JACOBSON of the Lexington Water Co. It indicates approximately its usual composition.

Color	< 5
pH	7.7
Turbidity	$< 20 = 1$
Total solids	191 ppm
Dissolved solids	191 ppm
Suspended solids	0
Cl	4 ppm
$CaCO_3$	93 ppm
Alkali $CaCO_3$	77 ppm
Alkali as OH	0
Alkali as HCO_3	77 ppm
Ca	26 ppm
Mg	7 ppm
SO_4	14 ppm
Fe^{+++}	0.1 ppm
Mn	0.0 ppm
Zn	0.0 ppm
Pb	0.0 ppm
Cr	0.0 ppm
Cu	0.0 ppm

The medium referred to in this report as S.G. medium was used by REILLY, HARRIS, and WAKSMAN (1947) for growing cultures of *Streptomyces griseus* (hence S.G.). It gave excellent growth with the yeast used in this study. Its composition is:

glucose	1.0 %
peptone	0.5 %
meat extract	0.5 %
sodium chloride	0.5 %
tap water	

The pH after sterilization in the autoclave at 15 lb. pressure for 15 min. was between 6.7 and 6.8. No adjustment was made. To make S.G. agar, 1.5 % Bacto agar was added.

The *Avena* coleoptile test used for auxin activity assay was essentially that given by BOYSEN-JENSEN (1936). The oat seeds were all *Avena sativa*, kindly provided from a single harvest by Dr. DAVID REID of the Department of Agronomy of the College of Agriculture at the University of Kentucky. This test was performed in a closed closet maintained at approximately 92 %

relative humidity. The only source of illumination reaching the oat seeds during the test came from a 60 watt frosted bulb encased in a cylinder containing 3 thicknesses of red cellophane.

The seeds were freed from their husks and were soaked in water for 2 to 3 hours. After this period they were laid out, groove downward, on wet filter paper in a Petri dish in the darkened closet. The seeds were allowed to germinate for about 30 hours, during which time they were intermittently exposed to the red light for a total of 15 minutes. The seedlings were then planted in glass holders of the type regularly used for this test (see WENT and THIMANN, 1937 ,p. 29), with the roots at the surface of tap water contained in a trough. After 48 hours the extreme tips of the seedlings were out off without cutting through the primary leaf. The primary leaves were then pulled so that they broke at the base and they were partially drawn out. Small blocks of agar, 40 × 20 × 20 mm, which had been impregnated with the compound to be tested, were then placed on one side of the cut surface of the leaf and held in place by capillarity. Ninety minutes after application of the agar blocks, the seedlings were photographed and the angle of curvature of each stem was measured with a protractor from photographic enlargements.

The greater the angle of positive curvature, the greater the auxin activity of the material in the agar blocks, within certain limits.

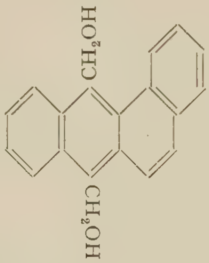
Unless indicated otherwise, all photomicrographs in this report were made in aqueous mount, unstained, and with the light microscope. The magnification indicated refers to the microscope magnification, but most of the photos have been magnified considerably beyond that by the use of a photographic enlarger during the printing of the positives.

Experimental

The Effect of 9 : 10-Bishydroxymethyl-1 : 2-benzanthracene (BB) on the Cell-Division Mechanism of *Sacchraromyces cerevisiae*

a) In S.G. broth cultures incubated with shaking

In an earlier study (SCHERR, unpublished data), the carcinogen 9 : 10-bishydroxymethyl-1 : 2-benzanthracene (BB) (I) was being used in an attempt to induce biochemical mutants in *S. cerevisiae*. The carcinogenic properties of this compound have been reported by BADGER and COOK (1940). It has been successfully used as a mutagenic agent by SCHERR and WEAVER (1949). Shelved in the laboratory for some time was a small centrifuge tube which contained a culture of *S. cerevisiae* in S.G. medium (see *Materials and Methods*) to which had been added BB to a final concentration of 0.204 mg/ml. A loopful from this culture, examined microscopically, showed the presence of large numbers of cells growing elongated in the form of filaments (fig. 4).



(I) 9:10—Bishydroxymethyl—1:2—benzanthracene

The entire sediment from this centrifuge tube was transferred to a 125 ml erlenmeyer flask containing 30 ml of S.G. broth and 5 ml of a stock carcinogen suspension (2.04 mg/ml in distilled water). This culture, designated 274D, was incubated at room temperature 22° C ±2°)[1] and examined periodically for 37 days. Microscopic studies of this and subsequent cultures were made in the following way.

The culture was well shaken by hand and 0.2 ml of the suspension, aseptically removed, was transferred to a 12 ml conical centrifuge tube, which was then spun for approximately 5 minutes at approximately 1500 rpm. The yeast cells are brought down with little difficulty. The supernatant was discarded and a portion of the sediment was transferred by loop to a slide and examined in wet mount under a cover glass. The results of the examinations made on culture 274D are described in table 1 and recorded photographically in fig. 5—10.

Not all of the yeast cells were visibly affected by the carcinogen in the transition from Y → F phase (fig. 8 and 9). Microscopic examination showed that the F forms occurred in packets, interspersed with normal-appearing budding yeast cells (fig. 5, 6, 10). This observation was particularly emphasized by LEVAN (1944), who, in referring to the camphor reaction, indicated that "it was found that the significant feature of the reaction was not so much the change in cell shape as the *hanging together in colonies of the cells*".

The older forms exhibited branching to a degree which made it difficult to ascertain which cells of the filament were the oldest (see, for eaxmple, fig. 6). It was also observed that inhibition of cell division[2] could occur without

TABLE 1. *Culture* 274*D of S. Cerevisiae exposed to* 9 : 10-*Bishydroxy-methyl-* 1 : 2-*Benzanthracene in S.G. Broth at Room Temperature*

Time of incubation (days)	Legend
8	Frequent strings of cells having the appearance of "racquet mycelium. Most of the cells, however, are typical budding yeast cells.
12	F forms more numerous, but still no more than approximately 10 % of culture.
15	F forms don't appear to be getting more numerous, but it appears that those which had been formed are increasing in length (fig. 5).
21	Trend of growth indicated after 15 days incubation appears to be substantiated. Note fig. 6, which represents a typical field.
26	No noticeable change in morphology. Fig. 8 shows a filament resembling a racquent mycelium.
37	Culture composed predominantly of F forms (fig. 10). Crystals of carcinogen still present.

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[1] The room temperature recorded here will not necessarily be identical with room temperatures reported in subsequent work because of seasonal changes occurring during the course of this study.

[2] In this report "cell division" will refer to the condition in which the daughter cell or bud becomes disassociated from the parent cell; cell multiplication will merely indicate the formation of a new cell and will be used, therefore, synonymously with reproduction. The term "growth" usually encompasses cell division and cell multiplication, but in the work on *S. cerevisiae* reported here a distinction is made among these three phenomena, since they can be separately assessed cytologically; e.g., the filamentous forms undergo growth and cell multiplication but are damaged in their cell division mechanism.

cell elongation taking place, so giving rise to strings of yeast phase cells. This is especially evident in fig. 7.

In order to confirm and extend the observations implicating BB in Y → F activity the following was performed.

The suspension of yeast cells to be used as an inoculum was prepared by inoculating *S. cerevisiae* into 30 ml of S.G. medium contained in a 125 ml erlenmeyer flask. This flask was then incubated at room temperature (23° C ± 2°) with shaking for 48 hours. Shaking was accomplished with a reciprocating shaker having 68 occillations per minute and a full stroke of 2½ inches. This culture was then centrifuged, the supernatant was discarded, and the residue cells were suspended in 25 ml of sterile buffered distilled water (BDW) (American Public Health Association, 1948). This suspension was disseminated in 0.1 ml amounts into 125 ml erlenmeyer flasks, each of which contained 27 ml amounts of S.G. medium and 3 ml amounts of a serial dilution of BB suspended in distilled water, to give a series of flasks containing final concentrations of 0.00002 to 0.204 mg/ml of BB. Table 2 outlines the method used. The control flask contained no carcinogen. The flasks were all incubated at room temperature with shaking. The aberrent forms were scored at 0 and after 2, 10, 22.5, 27, 99, 195, 315, and 555 hours of incubation. Plate clounts were made up to and including 27 hours of incubation.

In scoring the different types of aberrent forms found, the same technique was employed as previously outlined. The elongated cells which occur in chains were scored as filaments (F). Such filaments have been illustrated in fig. 9.

TABLE 2. *Serial Dilution of* 9 : 10-*Bishydroxymethyl*-1 : 2-*Benzanthracene in S.G. Broth Inoculated with Saccharomyces Cerevisiae*

S.G. Medium Flask	(ml)	BB in distilled water Dilution	(ml)	Final Conc. of BB in S.G. (Mg/ml)
1	27	stock	3	0.20400
2	27	1 : 10	3	0.02040
3	27	1 : 100	3	0.00204
4	27	1 : 1000	3	0.00020
5	27	1 : 10000	3	0.00002
6	30	—	0	0.00000

Although short filaments of cells were occasionally found (fig. 11), a culture of elongated cells of *S. cerevisiae* in which the inhibition of cell division was not also a concomitant phenomenon was not observed. The converse is by no means true, since the inhibition of cell division has been frequently observed without any accompanying cell elongation (fig. 12). I have called such a group of cells an "inflorescence" (Infl), using a term borrowed from the botanists. All cells scored as inflorescences fail to show evidence of cell elongation.

The relative number of inflorescences and filaments are estimated and recorded as follows:

—	none found
±	rare
+	few
++	frequent
+++	many
++++	very many

The results of this experiment are recorded in table 3 and plotted, in part, in the following graph. This graph only records the data up to and including 27 hours of incubation.

Filaments appeared in the cultures containing BB after 10 hours of incubation and increased in frequency up to the end of the experiment. Caution

TABLE 3. *The effect of* 9 : 10-*Bishydroxymethyl*-1 : 2-*Benzanthracene* (*BB*) *on Saccharomyces Cerevisiae in S.G. Medium incubated at room temperature with shaking*

Time of incubation (hours)	Conc. of BB (mg/ml)	Plate Count			Infl*	F**
		Dilution	No. of colonies	Count per ml		
0	0.00000[1]	10^4	32 20	2.6×10^5	—	—
2	0.20400	10^3	149 140	1.5×10^5	—	—
	0.02040	10^3	98 120	1.1×10^5	—	—
	0.00204	10^3	122 109	1.2×10^5	—	—
	0.00020	10^3	170 187	1.8×10^5	—	—
	0.00002	10^5	4 10	7.0×10^5	—	—
	0.00000	10^4	22 10	1.6×10^5	—	—
10	0.20400	10^5	54 31	4.3×10^6	—	±
	0.02040	10^5	22 102	6.2×10^6	±	+
	0.00204	10^5	28 35	3.2×10^6	±	+
	0.00020	10^5	46 31	3.9×10^6	±	+
	0.00002	10^5	83 99	9.1×10^6	—	—
	0.00000	10^5	61 59	6.0×10^6	—	—
22.5	0.20400	10^5	77 89	8.3×10^6	—	++
	0.02040	10^5	70 90	8.0×10^6	—	++
	0.00204	10^5	98 72	8.5×10^6	—	++
	0.00020	10^5	110 91	1.0×10^7	—	+

[1]) Counts at zero hours of incubation were determined only on the control. Since all flasks received equal aliquots of the cell suspensions of *S. cerevisiae* it is assumed that, within experimental error, equal numbers of cells were distributed.

* - Inflorescenses

** - Filaments

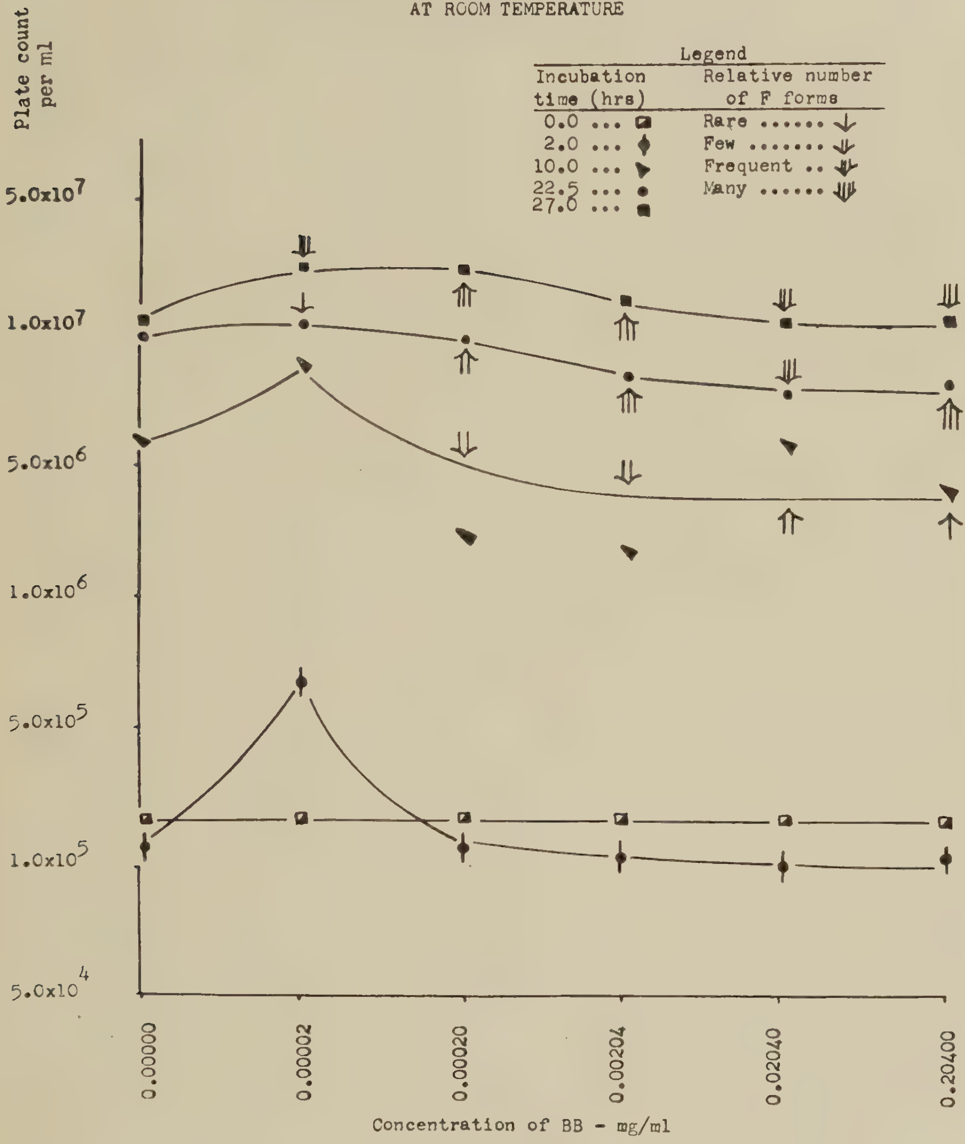
TABLE 3 (con'd)

Time of incubation (hours)	Conc. of BB (mg/ml)	Plate Count			Infl	F
		Dilution	No. of colonies	Count per ml		
	0.00002	10^5	163 141	1.5×10^7	—	±
	0.00000	10^5	131 110	1.2×10^7	—	—
27	0.20400	10^5	175 141	1.6×10^7	—	++
	0.02040	10^5	185 199	1.9×10^7	—	++
	0.00204	10^5	220 265	2.4×10^7	—	++
	0.00020	10^2	333 321	3.3×10^7	—	++
	0.00002	10^5	392 351	3.7×10^7	—	++
	0.00000	2×10^5	74 57	1.3×10^7	—	—
99[1]	0.20400				—	+
	0.02040				—	++
	0.00204				—	+++
	0.00020				—	+++
	0.00002				—	+++
	0.00000				—	±
195	0.20400				—	+
	0.02040				—	+
	0.00204				—	+
	0.00020				—	++
	0.00002				—	—
	0.00000				—	±
315	0.20400				—	+
	0.02040				—	++
	0.00204				—	++
	0.00020				—	—
	0.00002				—	++
	0.00000				—	++
555[2]	0.20400			Very large isolated clumps of F forms present. These clumps macroscopically visible and settle easily to the bottom of the flask where they appear as pellets about the size of the head of a pin. Normal-appearing yeast cells are still very much in evidence, with occasional asci being seen.		

[1] From this point all flasks were incubated at room temperature without shaking. Plate counts were discontinued.

[2] All flasks had been discarded except that containing 0.20400 mg/ml of BB.

must be used in interpreting the comparative effects of the different concentrations of BB, since its solubility is low. SCHERR (1949), using a technique developed by DAVIS, KRAHL, and CLOWES (1942), determined the solubility to be 0.00102 mg/ml in distilled water. The solubility in S.G. medium has not been determined. Note that beyond a certain concentration of the carcinogen (between 0.00020 and 0.00002 mg/ml), which is probably the point of maximum solubility in this medium, the relative number of filaments remained essentially the same. The low solubility of BB probably also results in an appreciable lapse of time between the addition of the carcinogen to the



THE EFFECT OF 9:10-BISHYDROXYMETHYL-1:2-BENZANTHRACENE ON SACCHAROMYCES CEREVISIAE IN S.G. MEDIUM INCUBATED AT ROOM TEMPERATURE medium and the point at which the soluble and insoluble fractions reach equilibrium. This factor was not evaluated. Undoubtedly the higher concentrations used in this experiment contained the same amount of soluble BB plus some of the undissolved compound. This is suggested by the straight line sections of the curves for the higher concentrations of BB for the various periods of incubation as indicated in the above graph.

After 2 hours of incubation the growth in the flask containing 0.00002 mg/ml of the carcinogen was nearly 5 times as great as in the control. With larger amounts of carcinogen and longer periods of incubation this stimulating effect decreased or disappeared. At this concentration of BB (0.00002 mg/ml) frequent filaments were not seen previous to the 27th hour of incubation. Topley and Wilson (1946) indicate that there is a concentration for nearly all salts, usually very low, which stimulates microbial growth. This phenomenon may be the explanation for the peak of growth found with the 0.00002 mg/ml concentration of BB after 2 hours of incubation.

Counts on the control culture and on the cultures containing higher concentrations of BB were slightly reduced after 2 hours of incubation, indicating death of some yeast cells, probably due to the shock of being placed in a new environment. The counts on the cultures containing the higher concentrations of BB remained slightly below those of the control after 10 and 22.5 hours of incubation but were slightly above those of the control after 27 hours.

Caution must be used in interpreting these data as indicating that the growth of the yeast was either inhibited or stimulated by the carcinogen BB. Each filament of yeast cells is composed of numerous cells and such a unit would, of course, give rise to a single colony; thus plate counts furnish a poor basis for comparing growth in cultures containing variable numbers of filaments. Attempts to break up the filaments by shaking were unsuccessful and attempts to separate the cells by micromanipulation indicated that they adhered to each other tenaciously. This observation was also made by Levan (1947) for cf-forms of yeast cells.

The highest incidence of F forms was found after 99 hours of incubation, yet many Y forms were still evident (fig. 13 and 14). Some decrease in F forms could be noted after 195 and 315 hours. Though it is possible that this decrease in the number of F forms after 99 hours of incubation is a real effect, this seems unlikely. Such a decrease could occur if the rate of growth of the F forms were to become markedly slower than that of the Y forms after a certain period of incubation, if the F forms were to autolyze at a faster rate than the Y forms, or if F → Y conversion were suddenly to occur. Any one of these possibilities would also require that the suggested mechanism be reversed sometime before 555 hours of incubation, since, at least for the single culture examined after this period of incubation, the number of F forms by far exceeded the number of Y forms, and F formation had progressed to a degree where microscopic pellets containing enmeshed filaments of cells could easily be detected. It seems more likely that the method employed here lacks the refinement of being able to distinguish differences of the order of + or even ++ consistently. A more refined method has been employed in some of the experiments to be described, but since the primary object in this work was to detect major differences in F formation induced by various agents, the method used here, requiring a minimum of time and labor, sufficed. Thus it has been used in most of the experiments recorded here.

As the age of the culture increased the F forms found were increasingly longer and fewer shorter filaments could be found. It appeared as if those filaments which had been formed at the onset of the experiment had become longer, while few new F forms were being induced with increased time of exposure to the carcinogen. This suggested that there might be a marked variation among the yeast cells in their susceptibility to the agent inducing the formation of F forms or that the F forms represented mutations which were induced during initial exposure of the cells to the carcinogen and that those cells in the yeast phase represented the progeny of the more resistant cells which had survived the initial shock of the toxic and mutagenic effects of the carcinogen. A fuller discussion of these possibilities will be presented later.

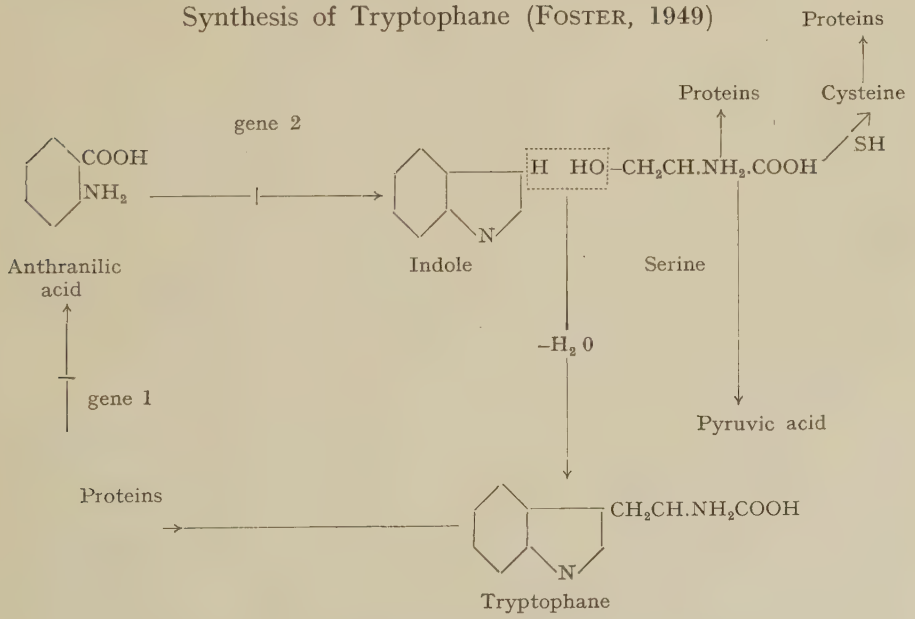
Giant forms of yeast cells became more prevalent in the cultures containing BB (fig. 15). Although these giant forms may also be found in control cultures containing no BB, their incidence appears to be enhanced by the carcinogen and by increased periods of incubation. These forms have not been scored in this work. Numerous other workers have recorded their occurrence under varied conditions (Dodge and Dodge, 1937; Subramaniam, 1945). Their designation as polyploids by Subramaniam may well be applicable here, but to determine this would require a genetic analysis.

The fact that gained immediate attention was the enhancement of cell elongation. It is important to note that F forms may be found in the control flask containing no BB after an extended period of incubation. This observation is in accord with those made by other workers whose papers have already been reviewed (Aderhold, 1894; Anderson, 1917), and will be demonstrated again in most of the experiments reported here.

That an increased concentration of auxin[1] may have been a possible cause of the elongated yeast cells was considered. Such an effect might be anticipated from work which will be briefly reviewed.

The work of Tatum and Bonner (1944) and Tatum, Bonner, and Beadle (1944) supports the view that tryptophane is synthesized as illustrated in the following scheme.

Synthesis of Tryptophane (Foster, 1949)



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[1] The term "auxin" will be used in this report to refer to those substances which bring about the specific growth reaction in which the degree of curvature of *Avena* coleoptiles is affected. This is in accord with the practice of the workers in the field of plant physiology.

If the action of BB were to make -SH groups unavailable for cysteine synthesis by some blocking action, then this would result in the accumulation of serine and tryptophane and a further shift in the direction of production of 3-indole acetic acid (heteroauxin), which stems from an oxidative deamination of tryptophane (THIMANN, 1935a; WILDMAN, FERRI, and BONNER, 1947; WILDMAN and MUIR, 1949). The accumulation of heteroauxin might result in the elongation of yeast cells, since elongation of plant cells is a characteristic effect of auxins (WENT and THIMANN, 1937). It is, in fact, the basis for the *Avena* test for auxin assay.

The specific interaction of carcinogenic compounds with the thiol groups of -SH activated enzymes was demonstrated by the researches of RONDONI (1948a, 1948b), RONDONI and BASSI (1948), and RONDONI and BARBIERI (1950). The digestion of gelatin by papain which is activated by cysteine or BAL (British Antilewisite) was partially inhibited by the carcinogen benzpyrene. The activating function of cysteine and BAL for cathepsins (autoproteolysins from horse liver) was completely suppressed by 9 carcinogenic compounds; 6 non-carcinogens did not inhibit autoproteolysis. CRABTREE (1944, 1945) was also able to show that certain substances which readily combine with -SH groups could partially inhibit the carcinogenic activity of chemical carcinogens. He showed that the carcinogenic action of 3,4-benzpyrene and 1 : 2 : 5 : 6-dibenzanthracene on mouse skin was greatly retarded by maleic and citraconic anhydrides. These anhydrides act by combining with -SH containing groups, and their interference with carcinogenicity functioned by interference with sulfur (S) metabolism. CRABTREE suggests that the primary phase of the carcinogenic process is the combination of carcinogens or their derivatives through S linkages to cell constituents. On the basis of considerations of the chemical reactivity of the carcinogenic hydrocarbons, WOOD and FIESER (1940) also suggested that a coupling between carcinogen and S-containing cell constituents was the first possible stage in their biological action.

The work of NICKERSON and VAN RIJ (1949) implicating -SH groups in the dimorphism mechanism of yeasts has already been mentioned.

The fact that auxin is elaborated by yeast cultures has been demonstrated by a number of workers (ROBINSON and STIER, 1940, 1941; WENT and THIMANN, 1937, p. 71). The work of KOGL and KOSTERMANS (1934) has shown that the auxin content of yeasts may be attributed almost entirely to 3-indole acetic acid. THIMANN (1935a, 1935b) has shown that the yield of auxin in cultures of *Rhizopus* was determined by the amount of tryptophane present. The reader is referred to the excellent monograph on the plant growth sybstances by WENT and THIMANN (1937) for a comprehensive presentation of this topic. A section on phytohormones is also contained in PINCUS and THIMANN's (1950) volume on *The Hormones*.

If the action of BB in enhancing Y → F formation were on a -SH grouping, then it would be expected that the addition of a sulfhydryl containing compound would antagonize the effect of BB. Accordingly, the following experiment was performed, utilizing cysteine as the sulfydryl-containing compound.

b) In synthetic medium with the addition of cysteine, stationary cultures.

For this experiment a synthetic medium, synthetic medium # 20, (see *Materials and Methods*) which is a modification of WICKERHAM's (1946) medium was used. Such a medium was used so that the quantity and type of S- and -SH containing compounds present during the course of the experiment would be known and controlled.

Into 6 tubes were measured 0.05 ml amounts of a stock suspension of

BB (2.04 mg/ml) in distilled water. To 3 of these tubes were also added 0.5 ml amounts of 0.1 molar 1 (+) cysteine hydrochloride (PFANSTIEHL). Medium # 20 was added to the experimental tubes to a final volume of 5 ml. Three control tubes were prepared to contain 5 ml of # 20 medium. To all 9 tubes were added 0.05 ml amounts of a suspension of *S. cerevisiae* cells prepared by harvesting the cells by centrifugation from a 24 hour culture grown in 30 ml of S.G. broth incubated at 25° C, washing them with BDW, and then suspending the cells in 10 ml of BDW. Tubes were prepared in triplicate so that incubation could be accomplished at 20°, 30°, and 37° C. The cultures were not shaken during incubation. The Infl and F forms were scored after 5 and 10 days of incubation in the manner previously described.

The amount of growth in the cultures was estimated by using a technique similar to that developed by WICKERHAM and BURTON (1948). By this method the culture is shaken and held before a white card upon which are drawn a number of parallel lines in India ink. The ease with which the lines are observed through the culture liquor is inversely proportional to the amount of growth and is arbitrarily rated as follows:

—	lines are distinctly seen
±	lines very slightly blurred
+	lines slightly blurred
++	lines very blurred
+++	lines barely seen
++++	lines obliterated

The results of this experiment are outlined in table 4.

The dimorphism effect (Y → F) was less pronounced in the # 20 medium stationary cultures than in the S.G. broth cultures incubated with shaking (table 3). The complete absence of F forms in all the tubes, even after 10 days of incubation, is in striking contrast to the previous experiment.

BB and cysteine in combination were more toxic than BB alone, a reaction which may be attributed almost solely to the action of the cysteine. The data are recorded in table 5a. The 0.01 molar concentration of cysteine at the 37° C incubation temperature proved to be more toxic alone than when it was used in conjunction with BB. This suggests that some of the cysteine may have been chemically bound by the BB, at least at 37° C. Here, too, no F forms were found.

A greater number of inflorescences (fig. 16) was found when BB and cysteine were used together than when either was used alone. This suggests that 0.01 molar cysteine may be too toxic to permit sufficient growth for the expression of inflorescences, but that when it is combined with BB some of the cysteine is oxidized (or neutralized in some other way) so that a more optimum concentration for Y → F activity results. A very large portion of the Y → F activity in the BB — cysteine mixture should be credited to cysteine, since BB alone in this medium showed little Y → F activity. The Y → F activity of cysteine presumably might be due to a shift in the tryptophane synthesis resulting from an upset equilibrium: cysteine → serine → tryptophane → heteroauxin.

Support for the idea that some neutralization of cysteine may be attributed to BB stems from the fact that when the concentration of cysteine was reduced tenfold to 0.001 molar, most of the toxic effects found when 0.01 molar concentration was used disappeared and a much stronger Y → F effect occurred (see table 5b). Also, when 0.01 molar cysteine was combined with 0.0204 mg/ml concentration of BB, the Y → F effect was comparable to that which was found with 0.001 molar cysteine alone (compare tables 4 and 5b).

TABLE 4. *The effect of* 9 : 10-*bishydroxymethyl*-1 : 2-*benzanthracene* (*BB*) *on the cell-division mechanism of Saccharomyces cerevisiae cultured in* # 20 *synthetic medium*

Incubation		Control			BB (0.0204 mg/ml)			BB (0.0204 mg/ml) + 1(+) cysteine (0.01 molar)		
Days	Temp (°C)	Growth	Infl	F	Growth	Infl	F	Growth	Infl	F
5	20	++++	—	—	+++	+	—	++	+++	—
	30	+++	—	—	+++	+	—	++	+++	—
	37	+++	—	—	+++	++	—	++	+++	—
10	20	++++	++	—	++++	++	—	++	++++	—
	30	++++	±	—	++++	+	—	++	++++	—
	37	++++	+	—	++++	++	—	+++	++++	—

TABLE 5a. *The effect of* 1(+) *cysteine on the cell-division mechanism of Saccharomyces cerevisiae cultured in* #20 *synthetic medium*

Incubation		Control			0.01 molar 1 (+) cysteine		
Days	Temp (°C)	Growth	Infl	F	Growth	Infl	F
5	20	++++	+	—	++	++	—
	30	++++	+	—	++	++	—
	37	++++	+	—	+[1]		
10	20	++++	+	—	++	++	—
	30	++++	+	—	+++	+++	—
	37	++++	+	—	+		

[1] No estimations of inflorescences and filaments were made if the amount of growth was below ++

It is possible that BB and cysteine both display Y → F activity due to different and possibly unrelated mechanisms and the combined effect is purely an additive phenomenon.

There is the possibility that BB may have been chemically inert when it was used in the # 20 medium cultures. This is unlikely, since there were such marked differences in inflorescence formation between the experiments with 0.01 molar cysteine alone and those in which it was in combination with 0.0204 mg/ml of BB.

The possibility that BB may have been physiologically inert for *S. cerevisiae* in the # 20 medium cultures is suggested by the fact that the inflorescence activity in #20 medium containing BB, when compared to controls lacking it, was very slight and bordered on the limits which could be graded as significant.

It is entirely possible that the more marked Y → F activity displayed by BB in S.G. broth may be attributed to one or more of the following:

TABLE 5b. *The effect of* 1(+) *cysteine and* 9 : 10-*bishydroxymethyl*-1 : 2-*benzanthracene* (*BB*) *on the cell-division mechanism of Saccharomyces cerevisiae cultured in* #20 *synthetic medium*

Incubation		Control			(1+) cysteine (0.001 molar)			BB (0.0204 mg/ml) + (1+) cysteine (0.001 molar)		
Days	Temp (°C)	Growth	Infl	F	Growth	Infl	F	Growth	Infl	F
5	20	++++	+	—	+++	++	±	++++	++	±
	30	+++	+	—	+++	+++	±	+++	+++	—
	37	+++	±	—	++	+++	—	++	+++	—
10	20	++++	++	—	++++	+++	±	++++	+++	±
	30	++++	++	—	++++	+++	±	+++	+++	—
	37	++++	++	—	++++	++++	—	+++	++++	±

1. The modification by BB in S.G. broth of -SH containing compounds outside of the yeast cell, which then enter the cell in their modified form and disrupt some biochemical system controlling the dimorphism mechanism. The synthetic medium #20 contains no -SH groups, but the S.G. broth no doubt does contain -SH compounds.

2. The modification of the -SH containing compounds outside of the cell, thus making them inaccessible to the yeast cell. This might require some modification in the cell's physiology in order to make up for a lack of preformed -SH groups.

3. A higher oxygen tension in cultures in S.G. broth incubated with shaking than in the 5 ml of synthetic medium in a test tube maintained in a stationary position. The formation of auxin from tryptophane proceeds through a biochemical pathway involving an oxidative deamination (THIMANN, 1935a; WENT and THIMANN, 1937).

The possibility of (1) is suggested by the facts that the synthetic medium used here contains no free -SH groups and that BB has little influence on Y → F formation in this medium. If BB were inducing Y → F formation in the S.G. broth by affecting some intracellular system directly, then it might be expected that the cell wall and/or the membrane of the yeast cell would not be impermeable to this carcinogen by dint of being grown in another medium, unless this other medium lacked some essential materials which might result in faulty synthesis of protoplasmic materials within the cell, or unless the composition of the medium itself were a direct factor in affecting this permeability.

The second possibility seems to have little foundation, since cells grown in #20 synthetic medium are not exposed to -SH groups and yet do not show Y → F formation.

The third possibility gets some support from the fact that cells grown in S.G. broth under the same conditions as used for the synthetic medium did not show as much Y → F activity as those in the S.G. broth cultures incubated with shaking. This knowledge was gained from the following experiments.

c) In S.G. broth stationary cultures

The experiment recorded here is a duplicate of the one described in section *b* in every respect except that S.G. broth was substituted for #20 synthetic medium. The results are outlined in tables 6 and 7.

The Y → F activity displayed by BB in stationary test tube cultures using S.G. broth was intermediate between that found in stationary test tube cultures using #20 synthetic medium and that found in S.G. broth cultures incubated with staking. These results cannot be explained entirely on the basis of oxygen tension unless it can be shown that the stationary cultures in S.G. medium had a higher oxygen tension than the cultures in #20 synthetic medium.

The enhanced Y → F activity found when BB and cysteine were used together in the synthetic medium also occurred in this experiment.

The increased F formation induced by BB in the S.G. broth stationary cultures, as compared to that in the synthetic medium cultures, strongly suggests that the presence of -SH groups in a medium can markedly modify the morphological disturbance induced by agents active in the production of Y → F transformations. The confirmation of this depends upon the demonstration of a direct correlation between the amount of -SH groups in a medium and the degree of Y → F activity induced in yeast cells by some agent.

If, as has been suggested by the data in tables 4 and 6, BB reacts in some way with cysteine, then it should be possible to demonstrate this aside from its effect on the growth of yeast cells. Since the most labile portion of the cysteine molecule is probably the -SH group, it was felt advisable to look for a change at this portion of the molecule first.

Table 6. *The effect of* 9 : 10-*bishydroxymethyl*-1 : 2-*benzanthracene* (*BB*) *on the cell-division mechanism of Saccharomyces cerevisiae cultured in tubes of S.G. broth*

Incubation		Control			BB (0.0204 mg/ml)			BB (0.0204 mg/ml) + 1(+) cysteine (0.01 molar)		
Days	Temp (°C)	Growth	Infl	F	Growth	Infl	F	Growth	Infl	F
5	20	++++	—	—	++++	±	—	++++	++	++
	30	++++	—	—	++++	+	±	+++	++	+
	37	++++	—	—	++++	+	+	++++	++	±
10	20	++++	—	+	++++	±	++	++++	++	+++
	30	++++	±	+	++++	+	+	++++	+	+++
	37	++++	—	+	++++	±	+	++++	++	++

d) The *in vitro* reaction between 9 : 10-bishydroxymethyl-1 : 2-benzanthracene and 1(+) cysteine in sterile #20 synthetic medium.

The method used for determining -SH groups was taken from Siggia (1949) and is a modification of the Kimball, Kramer, and Reid (1921) iodometric technique. This method utilizes a fixed amount of iodine to oxidize -SH groups, which are calculated by backtitrating the unused iodine with sodium thiosulfate, using starch solution as an indicator. The 0.1 N iodine, 0.05 N sodium thiosulfate, and starch indicator solutions were prepared according to the method of Hamilton and Simpson (1948).

The 0.1 N iodine solution was always added as a 10.00 ml quantity, using a burette, to the solution to be examined and back-titrated by burette with 0.05 N $Na_2S_2O_3$ solution, using a starch indicator. Although the standard procedure for the iodometric titration (Siggia, 1949) calls for 0.1 N $Na_2S_2O_3$

solution, it was hoped to gain more sensitivity by using 0.05 N. Since temperature corrections for the volume readings of the burettes used in this work were not made, every titration, both for the iodine and the $Na_2S_2O_3$ solutions, was begun at the 0.00 ml marking on the burette.

TABLE 7. *The effect of* 1(+) *cysteine and* 9 : 10-*bishydroxymethyl*-1 : 2-*benzanthracene* (*BB*) *on the cell-division mechanism of Saccharomyces cerevisiae cultured in tubes of S.G. broth*

Incubation		Control			1(+) cysteine (0.01 molar)			(0.001 molar)			BB (0.0204 mg/ml) + 1(+) cysteine (0.001 molar)		
Days	Temp ("C)	Growth	Infl	F	Growth	Infl	F	Growth	Infl	F	Growth	Infl	F
5	20	++++	—	—	++++	—	—	++++	—	—	++++	—	—
	30	++++	—	—	+++	+	—	++++	++	—	+++	+	—
	37	++++	—	—	++++	+	—	++++	±	—	++++	+	—
10	20	++++	—	+	++++	+	++	++++	+	+	++++	+	+
	30	++++	±	+	++++	+	+	++++	++	+	++++	++	+
	37	++++	—	+	++++	+	+	++++	+	+	++++	+	±

BB and cysteine, both singly and together, were added to sterile #20 synthetic medium and quantitative determinations of the residual -SH groups were made on 0.1 ml aliquots, using the iodometric technique, immediately before and after incubation for 20 hrs at 20° C and 37° C. In all cases the concentrations of BB and cysteine in the #20 medium were 0.0204 mg/ml and 0.01 molar, respectively.

Controles consisted of titrations of 10.0 ml of 0.1 N iodine and of 1.0 ml of #20 medium, The results are outlined in table 8.

The titres of 0.05 N $Na_2S_2O_3$ for the 10 ml amount of 0.1 N I_2 solution were essentially the same at the start of this experiment and after these solutions had remained at 20° for 20 hours. However, a 1.0 ml aliquot of sterile #20 medium gave a titre with $Na_2S_2O_3$ significantly higher than that for the 10 ml amount of 0.1 N I_2 alone, indicating that one or more oxidizing components in the #20 medium were being reduced by the $Na_2S_2O_3$. The titre for the #20 medium did not change after 20 hours of incubation at 20° C. The titre for the 10 ml amount of I_2 was 20.05 ml of $Na_2S_2O_3$, and for the 1 ml aliquot of # 20 medium plus 10 ml of I_2 it was 20.18 ml. It might, therefore, seem to be necessary to subtract the difference between these two values (00.13 ml) from all the other determinations for BB and cysteine in # 20 medium. This was not done because:

1. The 1 ml amount of # 20 medium when titrated alone is not exactly equal to 1 ml of # 20 medium when it contains BB and/or cysteine.

2. The primary objective of this experiment was to determine whether any oxidation of -SH groups could be attributed to BB. This information could be obtained independently of the titre for the # 20 medium. The purpose in titrating the # 20 medium was to determine if after 20 hours any change in its oxidation-reduction potentialities occurred which might interfere with the iodometric titration of -SH groups. Apparently, such was not the case under the conditions of this experiment.

The titre for cysteine alone was equal to that for cysteine plus BB, indicating that no oxidation of the cysteine by BB occurred, at least not in a quantity that could be detected by this method. The data, however, shows that the quantity of cysteine used could be detected by this method. Some oxidation of the cysteine occurred after 20 hours, the same quantity at both 20° C and 37° C.

TABLE 8. *The in vitro reaction between* 9 : 10-*bishydroxymethyl* 1 : 2-*benzanthracene* (*BB*) *and* 1(+) *cysteine in sterile* #20 *synthetic medium*

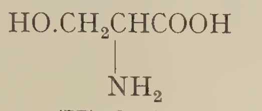
Tube No.	Legend [1]	Incubation		0.1 N I_2	0.05 N (ml)	$Na_2S_2O_3$ Average (ml)
		Temp °C	Hrs.			
1	Standardization			10.00	20.05 20.05	20.05
2	0.0204 mg/ml BB in #20 medium		0	10.00	20.00 20.05	20.03
4	0.01 molar cysteine in #20 medium		0	10.00	18.90 18.85	18.88
6	0.0204 mg/ml BB plus 0.01 molar cysteine in #20 medium		0	10.00	18.80 18.90	18.85
8	#20 medium		0	10.00	20.20 20.15	20.18
2	0.0202 mg/ml BB in #20 medium	20	20	10.00	20.05 19.95	20.00
3	0.0204 mg/ml BB in #20 medium	37	20	10.00	20.00 20.05	20.03
4	0.01 molar cysteine	20	20	10.00	19.10 19,10	19.10
5	0.01 molar cysteine in #20 medium	37	20	10.00	19.10 19.15	19.13
6	0.0204 mg/ml BB plus 0.01 molar cysteine in #20 medium	20	20	10.00	19.05 19.10	19.08
7	0.0204 mg/ml BB plus 0.01 molar cysteine in #20 medium	37	20	10.00	19.05 19.05	19.05
8	#20 medium	20	20	10.00	20.20 20.20	20.20
1	Standardization	20	20	10.00	20.00 20.05	20.03

[1] In every case a 1 ml aliquot of each sample was titrated in duplicate.

Some evidence, though admittedly circumstantial, that the reaction between BB and cysteine probably occurs at the -SH portion of the molecule can be gained from the data of the next experiment.

e) The effect of dl-serine and 9 : 10-bishydroxymethyl-1 : 2-benzanthracene (BB) on the cell-division mechanism of *Saccharomyces cerevisiae* cultured in # 20 synthetic medium.

If the reaction between BB and cysteine occurred at some point(s) of the cysteine molecule other than the -SH group, then it is possible that an effect similar to that found in the experiment outlined in section b (table 4) would occur between BB and serine (II), since serine differs from cysteine in structure only for the -SH portion of the cyteine molecule.



$$\mathrm{HO.CH_2\underset{\underset{NH_2}{|}}{C}HCOOH}$$

(II) Serine

Accordingly, the experiment outlined in section b was duplicated, using serine in place of cysteine. The only change made was that twice the quantity of serine was used, since the dl form was being used. The results of this experiment are outlined in tables 9 and 10.

The Y → F activity of the BB-serine mixture was less than the activity found in the BB-cysteine mixture under comparable experimental conditions (compare tables 4 and 9). In addition, the serine, used either alone or in conjunction with BB, was much less toxic to the yeast cells than cysteine was.

Serine showed Y → F activity greater than that found for 0.01 molar cysteine (compare tables 5a and 10), but the comparatively low Y → F activity of the 0.01 molar cysteine, as has already been mentioned, may be attributed to its toxicity. Thus, when 0.001 molar cysteine was used, the toxic effects were decreased and the Y → F activity was on a par with that found for serine (compare tables 5b and 10).

TABLE 9. *The effect of dl-serine and* 9 : 10-*bishydroxymethyl*-1 : 2-*benzanthracene* (*BB*) *on the cell-division mechanism of Saccharomyces cerevisiae cultured in* #20 *synthetic medium*

Incubation		Control			BB (0.0204 mg/ml)			BB (0.0204 mg/nl) + dl serine (0.02 molar)		
Days	Temp	Growth	Infl	F	Growth	Infl	F	Growth	Infl	F
5	20	++++	+	—	++++	+	—	++++	++	±
	30	++++	±	—	++++	+	—	++++	++	—
	37	++++	++	—	++++	++	—	+++	+++	—
10	20	++++	++	—	++++	++	—	++++	++	—
	30	++++	+	—	++++	+	—	++++	++	—
	37	++++	++	—	++++	++	—	+++	+++	—

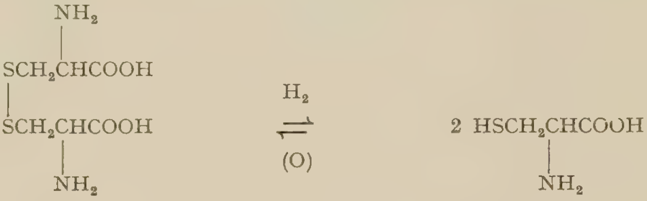
ABLE 10. *The effect of dl-serine on Saccharomyces cerevisiae in synthetic medium* # 20

Incubation		Control			0.02 molar dl serine		
Days	Temp	Growth	Infl	F	Growth	Infl	F
5	20	++++	+	—	++++	++++	—
	30	++++	+	—	++++	++	—
	37	++++	+	—	++	+++	—
10	20	++++	+	—	++++	+++	±
	30	++++	+	—	++++	+++	—
	37	++++	+	—	++++	++++	±

The essential difference between the molecules of cysteine and serine is that the -SH substituent in cysteine is replaced by an OH group in serine.

The toxicity of 0.01 molar cysteine may be due to some activity of the -SH group, or possibly to the activity of an oxidation product of cysteine (these two possibilities may be essentially the same thing). If the latter speculation is correct, the oxidation product of cysteine would not be serine, since 0.02 molar dl-serine displays very little toxicity when compared to cysteine.

The evidence for the presence of cysteine in proteins is indirect, resting with the identification of -SH groups in some proteins (FIESER and FIESER, 1944). FIESER and FIESER also indicate that the difficulty of isolating cysteine results from the ease with which it is readily oxidized to cystine.



$$\begin{array}{l}\overset{NH_2}{SCH_2CHCOOH} \\ \underset{NH_2}{SCH_2CHCOOH}\end{array} \underset{(O)}{\overset{H_2}{\rightleftharpoons}} 2\ \underset{NH_2}{HSCH_2CHCOOH}$$

Cystine Cysteine

Accordingly, it was felt advisable to examine the Y → F activity of cystine for *S. cerevisiae* in # 20 medium.

f) The effect of 1-cystine and 9 : 10-bishydroxymethyl-1 : 2-benzanthracene (BB) on the cell-division mechanism of *Saccharomyces cerevisiae* cultured in # 20 synthetic medium.

This experiment was a duplicate of the one using cysteine, which is described in section d, except that 1-cystine was used in place of 1-cysteine. The data are recorded in table 11.

TABLE 11. *The effect of* 1-*cystine and* 9 : 10-*bishydroxymethyl*-1 : 2-*benzanthracene* (*BB*) *on the cell-division mechanism of Saccharomyces cerevisiae cultured in* #20 *synthetic medium*

Incubation		Control			0.01 molar 1-cystine			BB (0.0204 mg/ml)			BB(0.0204 mg/ml) + 1-cystine (0.01 molar)		
Days	Temp (°C)	Growth	Infl	F	Growth	Infl	F	Growth	Infl	F	Growth	Infl	F
5	20	++++	—	—	++++	++	±	++++	++	±	++++	±	—
	30	++++	±	—	++++	++	±	++++	++	±	++++	+	—
	37	++++	—	—	+++	++	—	++++	+	—	++++	+	—
10	20	++++	±	—	++++	++	+	++++	++	++	++++	±	±
	30	++++	+	—	++++	+++	+	++++	++	+	++++	+	±
	37	++++	+	—	++++	+++	±	++++	+	±	++++	+	+

The .01 molar cystine was much less toxic for the yeast cells than was cysteine under comparable conditions (compare tables 5a and 11). Cystine also showed Y → F activity similar to that of cysteine and serine when their activities are compared using concentrations at which they displayed a low toxicity for the yeast cells (compare tables 5b, 10, and 11). The Y → F activity of cysteine, and of other agents, may be misleading when analysis is made at toxic levels, since it has not been determined whether the inflorescences are more or less resistant than Y phase cells to the toxic effects of the agent being tested.

BB showed somewhat higher Y → F activity than previously found under comparable conditions (compare tables 4, 9, and 11). It would appear that cystine was effective in antagonizing some of the Y → F activity of BB, though to a degree too small to be convincing. However, there definitely did not occur the enhanced Y → F activity found when 0.01 molar 1-cysteine (table 6) or .02 molar dl-serine (table 9) was added to BB. The data for cysteine, cystine, and serine do not readily lend themselves to an analysis of the part played by these compounds either alone or in conjunction with BB, in affecting the dimorphism mechanism of *S. cerevisiae.*

g) Discussion

There are a few inferences that can be made from the work on BB reported here. This carcinogen enhances the formation of filamentous forms of *S. cerevisiae* cells grown in S.G. broth cultures incubated with shaking; it enhances the formation of these forms to a lesser extent when the cultures are grown in a synthetic medium contained in test tubes kept stationary during incubation. That this difference in Y → F activity may be due to differences in oxygen tension in the media is suggested by the fact that the Y → F activity for BB in S.G. medium, when the experiment is performed in test tubes, is intermediate between that found in the test tube experiment using # 20 synthetic medium and that found in S.G. cultures incubated with shaking. This point will be discussed in greater detail later.

Two separate morphological entities of yeast cells can be distinguished, inflorescences and filamentous forms. That the cell elongation phenomenon may not be expressed until those cellular processes which give rise to inflorescences occur is suggested by the fact that single elongated cells have not been found and that inflorescences usually occur before filamentous forms, at least under the conditions of the experiments performed here (note, for example, tables 6, 7, 10 and 11).

One fact that stands out clearly in reports of studies on the mechanism of cell division is that -SH groups are involved (HAMMETT, 1930; GREGORY and CASTLE, 1931; GARDNER, 1940; PRATT and DUFRENOY, 1947; NICKERSON and EDWARDS, 1949; NICKERSON and VAN RIJ, 1949; THIMANN, 1949; O'CONNOR, 1950a). The results reported here lend some support to this fact in that BB plus cysteine gave greater Y → F activity than BB plus cystine or serine under comparable conditions (see tables 4, 5b, 9, and 11).

It has been postulated that agents which affect the cell-division mechanism probably act by upsetting a mechanism in which -SH groups are involved. In view of the objective of this study to attempt to arrest parasitic reproduction by inducing F or M formation of the yeastlike pathogens *in vivo*, two alternatives are suggested. One is the use of a substance which oxidizes the -SH groups required for cysteine synthesis, which should result in the accumulation of tryptophane and finally heteroauxin and thus, if auxin were the responsible factor, might induce F formation in the yeast phase cells. The other alternative is the use of auxins themselves in an attempt to affect the Y → F transition.

Since S- and -SH containing compounds are also essential to the metabolism of the parasitized host, it is to be expected that it would be very difficult to interfere with the -SH metabolism of the parasite at levels non-toxic to the host. Also, if it may be postulated that an interference with -SH metabolism disrupts the cell division mechanism, resulting in inflorescences, and this mishap is followed by a series of biochemical reactions which ultimately lead to the formation of auxin and a resulting effect of cell elongation, then it would appear that a simplified and more profitable approach to the induction of filament formation in *S. cerevisiae* might be to examine the properties of the auxins.

The Effect of Auxins on the Cell-Division Mechanism of Saccharomyces cerevisiae

a) The effect of alpha-naphthalene acetic acid (ANAA) on the cell-division mechanism of *S. cerevisiae* in S.G. broth

Alpha-naphthalene acetic acid (ANAA) is one of the substances having plant growth activity. Measured by the avena curvature test, it is approximately 1/40 as active biologically as 3-indole acetic acid; measured by the Pisum split stem curvature, it equals the latter in activity (WENT and THIMANN, 1937, p. 137). The difference in activity measured by these two tests is probably due to the fact that the difference in solubility of the two compounds is more manifest under the conditions of the avena test.

The effect of ANAA on the cell-division mechanism in *S. cerevisiae* was studied as follows:

One gram of commercial powdered ANAA (Kolker Chemical Co.) was dispensed into a tared sterile test tube, to which was added 10 ml of sterile distilled water. This constituted a stock suspension having a concentration of 0.1 g/ml. Two ml of this stock suspension were added to 18 ml of S.G. broth contained in a 125 ml erlenmeyer flask, a concentration of 10.0 mg/ml of ANAA. From this a series of serial dilutions were made in similar flasks of S.G. broth to give the following concentrations.

Flask #	Conc. of ANAA (mg/ml)
1	10.0
2	1.0
3	0.1
4	0.01
5	0.001
6	0.0001
7	0.00001
8 (control)	0.00000

Every flask was inoculated with a 0.1 ml amount of a 24 hour suspension of *S. cerevisiae* grown in S.G. broth and incubated at room temperature (22° C ± 2°) for 26 days. Scoring of F forms and inflorescences was made by the same method as in the previous experiments. The resultant data are compiled in table 12.

The 10.0 mg/ml concentration of ANAA is presumed to be fungicidal, since no growth occurred when the culture containing this amount was streaked out after 2 days of incubation, and no growth could be discerned in the flask even after 26 days of incubation.

After 4 days of incubation all the concentrations of ANAA that permitted growth enhanced the Y → F transition except the 0.0001 and 0.00001 mg/ml ones. After 26 days of incubation all the flasks in which growth occurred, including the control, showed Infl and F forms. The observation here that an extended period of incubation will result in Y → F activity among cells grown in a regular laboratory medium is in accord with that made in the experiments with BB.

TABLE 12. *The effect of alpha-naphthalene acetic acid (ANAA) on the cell-division mechanism of Saccharomyces cerevisiae in S.G. broth*

Time of incubation (days)	Conc. ANAA (mg/ml)	Growth	Infl	F	Remarks
2	10.0	—			Sterility of culture confirmed by streaking out on agar.
	1.0	++	—	—	
	0.1	++++	++	++	
	0.01	++++	++	—	
	0.001	++++	++	—	
	0.0001	++++	—	—	
	0.00001	++++	—	—	
	0.00000	++++	—	—	
4	10.0	—			Infl and F forms similar to those found with BB. Note fig. 18, 19, and 20. Fig. 20 is an unusual type of Infl.
	1.0	+++	++	+++	
	0.1	++++	+++	++	
	0.01	++++	++	—	
	0.001	++++	++	—	
	0.0001	++++	—	—	
	0.00001	++++	—	—	
	0.00000	++++	—	—	
26	10.0	—			
	1.0	++++	++	+++	
	0.1	(contaminated-discarded)			
	0.01	++++	+	+++	
	0.001	++++	+	+++	
	0.0001	++++	++	++	
	0.00001	++++	++	++	
	0.00000	++++	++	++	

The morphology of the Infl and F forms induced by ANAA was essentially the same as those induced by BB, except for one distinguishing characteristic. That is that many of the Infl induced by ANAA contained both typical yeast cells and elongated cells within the inflorescence (note fig. 17 and 19). Such an arrangement was not found in the course of the BB work. There was also the suggestion (fig. 20) that single elongated cells of *S. cerevisiae* were induced by ANAA, but this observation was made so infrequently that this possibility must await further work before it can be considered significant.

An examination of the inflorescences and filaments of the cells resulting from their exposure to ANAA and BB might well lead one to propose the explanation that the cell-division mechanism could not keep pace with growth and cell multiplication, so resulting in the formation of filaments. This explanation for the formation of filamentous cells was proposed by HINSHELWOOD (1946). Within this reasoning one would be forced to conclude that the effect of ANAA and BB on *S. cerevisiae* was to increase its rate of multiplication or delay the rate of cell division.

The delay of cell division has been readily accomplished in many organisms by irradiations. The increase in the size of cells following irradiation has been explained by LEA (1947) as the result, in most cases, of an inhibition of division while growth continues. Such an increase in size had been observed for bacteria (SPENCER, 1935; LEA, HAINES, and COULSON, 1937), yeast (HOLWECK and LACASSAGNE, 1930; WYCKOFF and LUYET, 1931), protozoa (MOTTRAM, 1926; ROBERTSON, 1933), fungal spores (LUYET, 1932), algae (LUYET, 1934), and other organisms (see Chap. VIII in LEA, 1947).

In order to attempt to assess the effect of the agents used here on the rate of growth (multiplication) of the yeast cells, the following experiment was performed.

b) The effect of alpha-naphthalene acetic acid (ANAA) on the growth rate of *S. cerevisiae* in S.G. broth

To 125 ml erlenmeyer flasks, each containing 18 ml of S.G. medium, were added 0.2 ml amounts of appropriate dilutions of the stock suspension of ANAA, prepared as previously described (section 3a) to yield a series of flasks having the following concentrations of ANAA in mg/ml: 10.0, 0.1, 0.001, and 0.00000 (control). The flasks of medium were inoculated as in the previous experiment and incubated at 20° C for 48 hours. Plate counts were made immediately after inoculation of *S. cerevisiae* into the culture flasks and after 12 and 24 hours; microscopic counts were made after 12 and 24 hours. The direct microscopic counts of cells and of inflorescences and filaments of cells were made in duplicate using a Hellige improved Neubauer Haemacytometer under 440 magnification. Counts of individual cells and of filaments of various lengths were made. An attempt to make another series of counts after 48 hours of incubation failed because it was not possible to accurately count the large numbers of cells present in many of the larger filaments and inflorescences.

The data in table 13a represent the average of the duplicate counts. The table includes summaries of the number of cells and number of clones per ml. The term clone is used here to refer to the total of individual cells plus filaments, a figure which should correspond with the plate count if all the cells are viable. Plate count results are recorded in table 13b.

The 10.0 mg/ml concentration of ANAA was germicidal, sterilizing the culture within 12 hours. The 0.1 and 0.001 mg/ml concentrations appreciably stimulated the production of filaments. As judged by the microscopic method the 0.1 mg/ml concentration resulted in a moderate increase in number of cells produced. The plate counts of the lower concetrations of ANAA, however, were approximately 40 per cent below that of the control. The small size of the plate counts in proportion to the microscopic counts indicates that many of the cells in the cultures were non-viable. The greater differences with the ANAA cultures indicate that all the concentriations of ANAA displayed some toxicity.

The interpretation of results in an attempt to determine whether the effect of the ANAA was merely to inhibit cell division, or whether it also modified the rate of cell multiplication, is difficult. While the number of cells produced by the 0.1 mg/ml concentration was apparently increased, the plate count results show that the culture contained less viable cells. It appears that the increased multiplication may have resulted from less competition with viable metabolizing cells. One is led to believe then that the effect of ANAA on *S. cerevisiae* is to modify the cell division mechanism without any appreciable change in the rate of cell multiplication.

The work of numerous investigators has indicated that auxin permits growth by increasing the plasticity of cells, so that elongation of plant cells

TABLE 13a. *The effect of alpha-naphthalene acetic acid (ANAA) on Saccharomyces cerevisiae in S.G. medium at 20: C*

Incubation time	Conc. ANAA mg/ml		Number of cells per filament																												Total cells per ml	Total clones per ml	Mean filament length
hrs			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	20	21	24	26	28	29	30	32	44	45	x10⁶	x10⁶	
12	10.0	No. of filaments	3	2	1			1																							16	7	2.3
	0.1		3	9	5	9	4	6	1	4	3	2	1				1	1													256	49	5.2
	0.001				3	5	4	2	2	2				1																	103	19	5.4
	0.00			14	3	9	2	6	1	1																					134	36	3.7
24	10.0		2	6	1																										17	9	1.9
			5	243	21	88	15	16	7	7	4	3	1	3		1				2		1			1	1	1	1	1	1	1494	403	3.7
	0.001		6	142	14	50	29	17	8	11			1		2			1	1		1		1	1		1					1092	286	3.8
	0.00			135	59	78	21	7	3	1	2			1	1																978	308	3.1

1) The term clone refers to individual cells and to filaments of cells.

may occur with no corresponding increase in weight (see WENT and THIMANN, 1937, for a comprehensive discussion of this topic.) The demonstration that ANAA induced Y → F formation without any significant change in cell capital (mass) requires for its support the demonstration that the average ANAA-induced filamentous cell of *S. cerevisiae* has the same mass as the average yeast phase cell. This could best be accomplished with cultures containing a high percentage of F forms (over 90 %), induced, preferably, by an agent having a minimum toxic effect on the culture. At the present time this has not been feasible.

TABLE 13b. *The effect of alpha-naphthalene acetic acid (ANAA) on Saccharomyces cerevisiae in S.G. medium at* 20° C

Incubation time (hr.)	Conc. ANAA (mg/ml)	Plate count (per ml)
0	all flasks	1.1×10^5
12	10.0	sterile
	0.1	2.5×10^5
	0.001	1.2×10^5
	control	2.5×10^5
24	10.0	sterile
	0.1	5.3×10^6
	0.001	5.2×10^6
	control	9.8×10^6

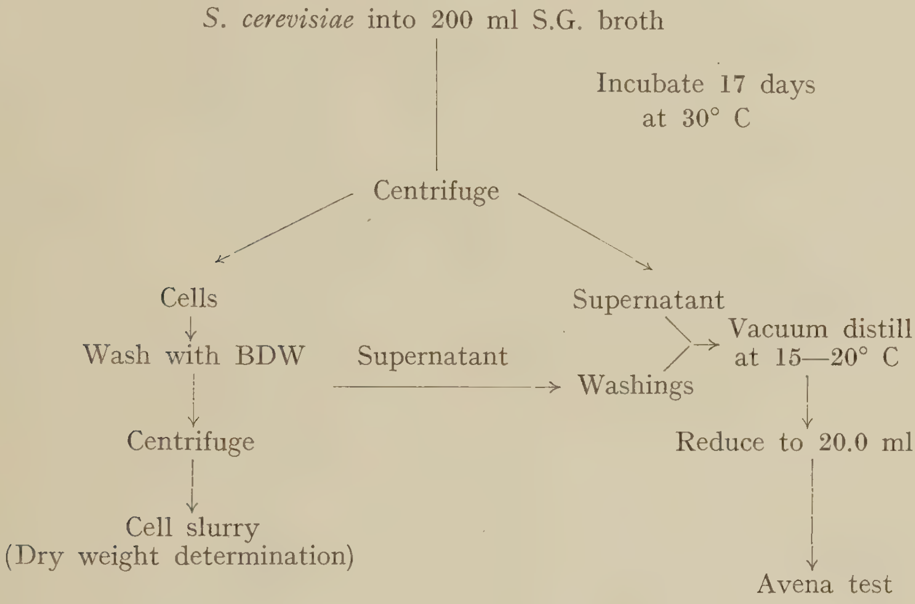
A correlation between prevalence of F forms and auxin content has, however, been shown. It has been possible to demonstrate that the auxin activity of a filtrate from cultures having a relatively large number of F forms of *S. cerevisiae* is greater than the auxin activity of a filtrate from a yeast phase culture, with both cultures containing comparable weights of cells.

c) The auxin activity of filtrates from cultures containing yeast and filamentous forms of *S. cerevisiae.*

Since it was desirable to examine the auxin activity, using the avena test, of filtrates from cultures containing F forms of *S. cerevisiae*, it was inadvisable to use a compound having auxin properties to induce the F forms. The use of BB was decided upon because auxin activity for BB could not be demonstrated with the avena test; BB at some of the higher concentrations used did not display any discernible toxicity above that which could probably be attributed to the shock of cells which frequently precedes the lag phase; and the concentrations of BB which exhibited Y → F activity for *S. cerevisiae* in S.G. broth were small enough so as not to interfere with weight measurements.

To each of two 1-liter erlenmeyer flasks were added 200 ml of S.G. broth. These flasks were autoclaved at 15 lb. pressure for 15 minutes. To one of these flasks was added 2 ml of a stock suspension of BB, to yield a final concentration of 0.0204 mg/ml. Both flasks were inoculated with 0.1 ml amounts of a suspension of cells of *S. cerevisiae* in buffered distilled water (BDW) harvested from 48 hour culture grown on an S.G. agar slant, and incubated at 30° C for 17 days. At the end of this period the numbers of filaments and inflorescences were determined for each culture.

Both cultures were then processed in the following manner. The culture was centrifuged and the supernatant liquid was separated from the cells by decantation. The cells were washed with approximately 50 ml of BDW and this washing was added to the supernatant liquid. The volume of the slurry of cells was measured ,and the dry weight of cells in this slurry was determined by weighing an amount of this slurry in a tared weighing boat and then drying it for 24 hours in an oven set at 110° C. The supernatant liquid plus cell washings was then reduced to a 20.0 ml volume by vacuum distillation at a temperature between 15° and 20° C. One ml of the reduced supernatant liquid was mixed with 1 ml of 3 % agar and the auxin activity was determined as previously described (see *Materials and Methods*). This process is outlined in the following scheme.


S. cerevisiae into 200 ml S.G. broth
Incubate 17 days at 30° C
Centrifuge
Cells
Supernatant
Wash with BDW
Supernatant
Vacuum distill at 15—20° C
Washings
Centrifuge
Reduce to 20.0 ml
Cell slurry (Dry weight determination)
Avena test

The data for this experiment are recorded in table 14. The filtrate from the culture which contained the greater number of F forms had a significantly greater auxin activity than that of the control. This observation confirms that of Robinson and Stier (1941). Although it is not possible to determine from the data of this experiment that elongation of cells was not accompanied by an increase in weight, some support is gained for this idea from the fact that the total dry weights of cells in the S.G. broth and in the broth containing BB did not differ by more than approximately 8 %. This could be still more significant if we draw on the data in tables 13a and 13b to reason by analogy that the rate of cell multiplication was not significantly changed.

Since a relationship between auxins and Y → F activity was becoming more evident, further auxins were tested for their activity.

d) The effect of beta-3-indolepropionic acid (IPA) on the cell-division mechanism of *S. cerevisiae*

Beta-3-indolepropionic acid (IPA) is a plant growth regulator and, with 3-indole acetic acid, has been proposed as part of the scheme for tryptophane breakdown (Porter, 1946). The auxin axtivity of IPA is approximately

1/1000 of the activity of auxin a, auxin b, and 3-indole acetic acid (see table III in Went and Thimann, 1937).

Table 14. *The auxin activity of filtrates from cultures containing yeast and filamentous forms of Saccharomyces cerevisiae*

		S.G. broth	S.G. broth containing 0.0204 mg/ml BB
Y → F activity:	Growth	++++	+++
	Infl	+	++
	F	±	++
Cells: Vol. of slurry		13.0 ml	30.0 ml
	Gross	51.9431 g	53.5798 g
	Tare	48.5818 g	48.5714 g
Slurry aliquot	Net	3.3813 g	5.0084 g
After 24 hr. at 110° C	Gross	48.6442 g	48.6149 g
	Tare	48.5818 g	48.5714 g
	Net	0.0624 g	0.0435 g
% dry wt. of cell slurry		1.86	0.87
Total dry wt. of cell slurry (Total cells in culture)		0.242 g	0.261 g
Avena test: coleoptile curvatures		+15.0°	
		+ 9.0°	+25.0°
		+ 8.0°	+22.0°
		+11.0°	+17.0°
		+12.0°	+15.0°
		55.0°	79.0°
Mean		+11.0°	+19.8°
Average dev. from mean		± 2.0°	± 3.8°

Five mg of powdered commercial IPA, kindly provided by the DOW Chemical Co., were suspended in 5 ml of #20 synthetic medium contained in a sterile test tube. Serial dilutions were then made, using #20 medium, to yield a series of tubes ranging in concentration of IPA from 1.0 to 0.0001 mg/ml. A control tube contained no IPA. These tubes were inoculated with 0.05 ml amounts of a washed cell suspension of *S. cerevisiae*, incubated at 25° C, and examined periodically for Y → F activity. The results are outlined in table 15.

The IPA did not enhance the Y → F activity to any significant degree in the #20 synthetic medium.

The activity of IPA was also studied in S.G. broth cultures incubated with shaking, by the method previously described. The data for this are recorded in table 16.

The Y → F activity of the IPA in the S.G. broth cultures that were shaken was higher than that found in the #20 synthetic medium.Although F forms did not appear until after 14 days of incubation, the elongated cells were much longer than those found in the previous studies, averaging 2 to 3 times the

TABLE 15. *The effect of β-3-indolepropionic acid (IPA) on the cell-division mechanism of Saccharomyces cerevisiae in #20 synthetic medium at 20° C*

Incubation (days)	Conc. IPA (mg/ml)	Growth	Infl	F
5	1.0	+++	+++	—
	0.1	++++	++	—
	0.01	++++	++	—
	0.001	++++	++	—
	0.0001	++++	++	—
	0.000	++++	++	—
10	1.0	++++	+++	—
	0.1	++++	++	—
	0.01	++++	++	—
	0.001	++++	++	—
	0.0001	++++	++	—
	0.000	++++	++	—

TABLE 16. *The effect of β-3-indolepropionic acid (IPA) on the cell-division mechanism of Saccharomyces cerevisiae in S.G. broth cultures incubated at room temperature with shaking*

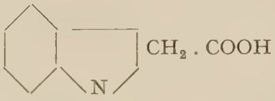
Incubation (days)	Conc. IPA (mg/ml)	Growth	Infl	F
2	10.0	++	+++	—
	1.0	++++	++	—
	0.1	++++	+	—
	0.01	++++	±	—
	0.001	++++	±	—
	0.0001	++++	±	—
	0.00001	++++	±	—
	0.000	++++	±	—
4	10.0	+++	++	—
	1.0	++++	±	—
	0.1	++++	±	—
	0.01	++++	±	—
	0.001	++++	±	—
	0.0001	++++	±	—
	0.00001	++++	±	—
	0.000	++++	±	—
14	10.0	+++	++	—
	1.0	++++	++	++[1]
	0.1	++++	++	++
	0.01	++++	++	±
	0.001	++++	++	±
	0.0001	++++	+	±
	0.00001	++++	±	±
	0.000	++++	±	±

¹) Elongated cells were much longer than any previously found.

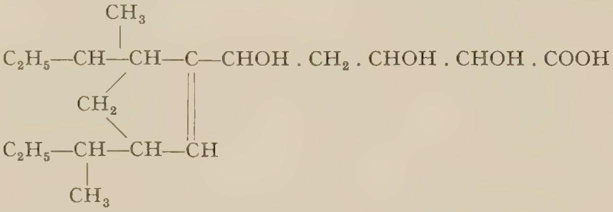
length of those shown, for example, in fig. 6. These very long cells were found in the cultures containing 1.0, 0.1, and 0.01 mg/ml of IPA, so that 0.01 mg/ml might be considered close to the limit of physiological activity for this compound under the conditions of this experiment. The concentration of 10.0 mg/ml was visibly toxic to the cells and did not result in F formation.

e) The effect of 3-indole acetic acid (IAA) on the cell-division mechanism of *S. cerevisiae*

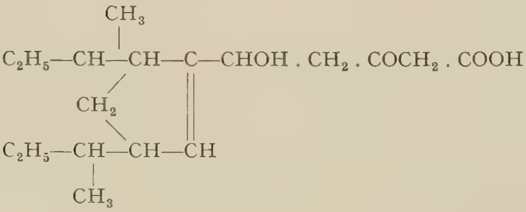
The isolation of 3-indole acetic acid (III) from yeast cultures and urine was accomplished by SALKOWSKI in 1885(b), but it was not until 1934 that KOGL, HAAGEN-SMIT, and ERXLEBEN, reisolating this substance from urine, demonstrated its auxin activity. It was then named "heteroauxin", the term usually used at the present time in referring to it. The activity of 3-indole acetic acid (IAA) is of the same order as that of auxin a (IV) and auxin b (V), and these three compounds are among those showing the highest auxin activity as judged by the avena curvature test (WENT and THIMANN, 1937, p. 137).



(III) 3-Indole acetic acid (heteroauxin)



(IV) Auxin a (Auxentriolic acid)



(V) Auxin b (auxenolonic acid)

The Y → F activity of IAA on *S. cerevisiae* was tested exactly as described for IPA in the S.G. broth cultures (see previous section d). The results are recorded in table 17.

The amount of Y → F activity displayed by IAA was small and the time required for its expression relatively long, when compared to what would be expected, considering its known auxin activity, if auxin activity were responsible for cell elongation. A repetition of this experiment using tubes of #20 synthetic medium produced little or no enhancement of Y → F activity (table 18).

One of the major difficulties of the work reported here on the Y → F activity of various agents stems from the fact that many of the chemicals examined are relatively insoluble in the different media used. This is true for BB, ANAA, IPA, and IAA. A more acceptable decision as to the Y → F activity of these compounds awaits their examination under conditions in which they are in true solution (not mechanical suspension or even colloidal dispersion).

TABLE 17. *The effect of* 3-*indole acetic acid* (*IAA*) *on the cell-division mechanism of Saccharomyces cerevisiae in S.G. broth cultures incubated with shaking at room temperature*

Incubation (days)	Conc. IAA (mg/ml)	Growth	Infl	F
2	10.0	+		
	1.0	++++	±	—
	0.1	++++	±	—
	0.01	++++	±	—
	0.001	++++	±	—
	0.0001	++++	±	—
	0.00001	++++	±	—
	0.000	++++	±	—
4	10.0	++	++	—
	1.0	++++	+	—
	0.1	++++	+	—
	0.01	++++	+	—
	0.001	++++	+	—
	0.0001	++++	+	—
	0.00001	++++	+	—
	0.000	++++	±	—
14	10.0	++++	+++	+
	1.0	++++	++	+
	0.1	++++	++	+
	0.01	++++	++	±
	0.001	++++	++	±
	0.0001	++++	+	±
	0.00001	++++	+	±
	0.000	++++	+	±

TABLE 18. *The effect of* 3-*indole acetic acid* (*IAA*) *on the cell-division mechanism of Saccharomyces cerevisiae in* #20 *synthetic medium at* 25° *C*

Incubation (days)	Conc. IAA (mg/ml)	Growth	Infl	F
5	1.0	+++	++++	—
	0.1	++++	+++	—
	0.01	++++	++	—
	0.001	++++	++	—
	0.00	++++	++	—
10	1.0	+++	+++	—
	0.1	++++	++	—
	0.01	++++	++	—
	0.001	++++	++	—
	0.00	++++	++	—

If a soluble precursor of heteroauxin were added to a culture of *S. cerevisiae*, then any Y → F activity which might occur might be associated with auxin formation. Since the work of many investigators has already established that tryptophane is a precursor of 3-indole acetic acid, this compound was next examined for Y → F activity.

f) The effect of tryptophane on the cell-division mechanism of *S. cerevisiae*. A stock solution of 0.1 molar 1-tryptophane (Nutritional Biochemicals Corp.) was prepared by suspending 0.204 g of the commercial crystalline compound in 10 ml of sterile distilled water and, while the solution was warmed to approximately 50° C, adding dropwise an amount of 0.1 normal HCl just sufficient to solubilize the tryptophane. Five tenth ml aliquots of this stock solution of tryptophane were aseptically added to 4.5 ml amounts of #20 synthetic medium. These tubes were inoculated with 0.05 ml amounts of a washed cell suspension of *S. cerevisiae* and incubated at 20° C, 30° C, and 37° C. Y → F activity was assayed after 5 and 10 days of incubation. Table 19 contains the data for this experiment.

Table 19. *The effect of* 1-*tryptophane solubilized as the hydrochloride on the cell-division mechanism of Saccharomyces cerevisiae in* # 20 *synthetic medium*

Incubation		Control		F	0.01 molar 1-tryptophane		F
Days	Temp (°C)	Growth	Infl		Growth	Infl	
5	20	++++	+	—	++++	++++	++
	30	++++	±	—	++++	++	—
	37	++++	++	—	++++	++	—
10	20	++++	++	—	++++	++++	++
	30	++++	+	—	++++	++++	±
	37	++++	++	—	++++	+++	—

The 0.01 molar tryptophane elicited a strong Y → F reaction in the culture after 5 days of incubation, the strongest activity being found at 20° C. There was a slight increase in Y → F activity after 10 days of incubation over that found after 5 days. The filamentous cells did not resemble those found in previous experiments but resembled germinating yeast phase cells going into hyphael formation (fig. 21).

An attempt was made to determine the concentration of tryptophane required for Y → F activity, using the method described above except that the tryptophane was added to the #20 synthetic medium in the form of a mechanical suspension, no HCl being added to solubilize the tryptophane. Under these conditions, although some Y → F activity was indicated by the presence of inflorescences, no filaments of the type illustrated in fig. 21 could be discovered (see table 20). Nevertheless, no crystals of tryptophane could be found in the cultures, indicating that it had all gone into solution. The effect of acids on the growth of plant tissues is especially pertinent and a portion of Went and Thimann's (1937, p. 130) discussion of this topic is quoted here.

"Bonner (1934) has shown . . . that . . . the growth caused by acid . . . increases steadily towards higher acidities. If we allow for the fact that the pH within the tissues is not the same as the pH of the (acid) buffer, then the curve of acid growth against pH follows closely the pH dissociation curve of auxin (pK = 4.8). This suggests that only the free acid form of auxin has growth-promoting activity, and that the applied acid sets free the auxin acid from the salt form in which it is present (pH of sap = 6.1). That the applied acid is not in itself effective in producing growth is supported by the fact that 2 hours after decapitation, when the auxin content has fallen to about one half . . . the curvatures produced by acid are also reduced to about one half. It is still more clearly borne out by the experiments of (THIMANN, 1935c). He immersed coleoptile sections in acid buffer, which caused temporary growth acceleration: when this had ceased, immersion in fresh acid had no effect because all the auxin in the sections had been used up, but immersion in auxin gave good growth.

Thus it is clear that the action of acid is *through its effect on the auxin in the plant . . .*"

TABLE 20. *The effect of* 1-*tryphophane on the cell-division mechanism of Saccharomyces cerevisiae in* # 20 *synthetic medium*

Incubation		Control			1-Tryptophane					
					0.01 molar			0.001 molar		
Days	Temp (°C)	Growth	Infl	F	Growth	Infl	F	Growth	Infl	F
5	20	++++	+	—	++++	++	—	++++	+++	—
	30	++++	+	—	++++	+	—	++++	++	—
	37	++++	+	—	++++	+++	—	+++	+++	—
10	20	++++	+	—	++++	++	—	++++	++	—
	30	++++	+	—	++++	+	—	++++	++	—
	37	++++	+	—	++++	++	—	++++	+++	—

It was therefore necessary to ascertain whether the quantity of HCl required to solubilize the tryptophane would cause the same Y → F activity in the absence of tryptophane. This was done as follows:

Ten ml quantities of #20 medium containing 0.01 molar 1-tryptophane were aseptically added to 5 sterile 125 ml erlenmeyer flasks. To this series of flasks, and to another set which lacked only the tryptophane, were added various quantities of N/1 HCl or N/1 NaOH in order to adjust the pH of the medium to various levels. The pH of the unadjusted medium was 6.65. All the flasks were inoculated with 0.1 ml amounts of a washed cell suspension of *S. cerevisiae* and incubated at room temperature (20° C ± 2°) with shaking. The results are compiled in table 21.

TABLE 21. *The effect of pH on the Y → F activity of* 1-*tryptophane on Saccharomyces cerevisiae in* # 20 *synthetic medium, incubated at room temperature for* 48 *hours*

Control				0.01 molar 1-tryptophane			
pH	Growth	Infl	F	pH	Growth	Infl	F
2.70	++	++	—	2.65	++	+++	—
3.15	+++	++	—	3.10	+++	++++	+
6.65[1]	++++	+	—	6.50[1]	++++	++	—
8.28	+++	±	—	8.25	+++	±	—
9.00	++	±	—	9.05	++	±	—

[1] pH of these cultures was unadjusted.

Although the effect of acid was to increase the number of inflorescences compared to those found in the unadjusted culture, the acid plus tryptophane yielded the highest Y → F activity, the maximum activity being found at a pH of 3.10. It is significant that at the alkaline pH values, irregardless of the presence of tryptophane, only negligible numbers of inflorescences and no F forms were found.

These data are in accord with the hypothesis that 1-tryptophane was utilized by the yeast as the precuror of heteroauxin and that the role of a low pH was to release the free indole acetic acid compound from an inactive complex, so that its activity on cells of *S. cerevisiae* became evident.

The Reversibility and Stability of the Y → F Reaction

One of the observations recorded by Levan (1947) as a characteristic of the cf-reaction was that the reaction is reversible. That is, if the concentration of the active compound in the medium falls below its threshold value for cf-activity, then cf-cells start to bud off normal-appearing daughter cells, which in turn behave normally. This assumes that the period required to reach the peak of cf-activity is not of such duration as to exhaust the essential nutriments required for cell multiplication.

The following experiment was performed in order to ascertain whether such an effect of "reversibility" can be found in cultures where Y → F formation has been induced. When the experiment with ANAA was being run, 0.1 ml aliquots were removed from the cultures containing the 1.0 and 0.01 mg/ml concentrations of ANAA after 26 days of incubation (see table 12) and streaked on plates containing S.G. agar, in duplicate. These plates were incubated at room temperature for 3 days. No colonies were found that indicated that an Infl or F of cells had bred true. No rough colonies were found and, of the approximately 20 colonies picked at random and examined microscopically, none showed any significant Y → F activity. It was thus established that the vast majority of the Infl and F forms revert to normal growth when the Y → F active agent is depleted.

On the other hand, when yeast phase cells of *S. cerevisiae* were inoculated onto plates of S.G. agar containing 1 mg/ml of ANAA, a characteristic change in colonial and cell morphology occurred. This experiment was performed as follows:

Three plates were poured with S.G. agar containing 1 mg/ml of ANAA. A set of control plates contained S.G. agar but no ANAA. To each plate was added a 0.1 ml amount of an 8 hour culture of *S. cerevisiae* in S.G. broth, which was streaked out. The plates were incubated at 20°, 30°, and 37° C for 8 days, one of each set at each temperature. The colonies were examined microscopically for cell morphology. The results are outlined in table 22.

That the ANAA was toxic to the yeast cells was shown by the diminutive size of the colonies (compare fig. 25 and 26 with fig. 22, 23, 24). There was also pronounced Infl formation at 30° C. No growth occurred at 37°.

One of the colonies from the ANAA-containing plate incubated at 20° C was streaked out onto a fresh S.G. agar plate containing the same concentration of ANAA. This procedure was also carried out with a colony from the ANAA-containing plate incubated at 30° C. The inoculated plates were incubated at the same temperatures at which their sources of inocula had been incubated, again for a period of 8 days. It was found that the growth of *S. cerevisiae* was extremely poor after this transfer and that most of the colonies were of pin-point size. The cell morphology still showed approximately +++ Infl and + F forms in both plates. Certainly the cells had not become adapted to the toxic effects of ANAA by this serial transfer using an ANAA-containing medium.

The marked toxicity displayed by the ANAA after a serial transfer was made probably indicates that the Y → F effect is limited, i.e., that Infl and

F forms cannot be further enhanced by increasing the concentration of the Y → F agent beyond a certain level, at which toxic effects become manifest and prevent growth completely. These data are in accord with the observation for auxins, that small concentrations may enhance growth (and cell elongation), whereas higher concentrations will prevent growth (FIEDLER, 1936).

TABLE 22. *The effect of* 1.0 *mg/ml alpha-naphthalene acetic acid (ANAA) in S.G. agar on the growth of Saccharomyces cerevisiae after* 8 *days incubation.*

	Incubation Temp. (°C)	Remarks
Control, no ANAA	20	Typical yeast colonial growth (fig. 22); budding cells only
	30	Same as above (fig. 23)
	37	Same as above (fig. 24)
1.0 mg/ml ANAA in S.G. agar	20	Minute colonies, appear dull (fig. 25); Infl +, F −
	30	Same as above (fig. 26); Infl ++++, F −
	37	Sterile

Further evidence that Infl and F are not stable forms resulting from their concentration by selection is gained from the following experiment.

TABLE 23. *The stability of inflorescences and filamentous cells of Saccharomyces cerevisiae induced by* 9 : 10-*bishydroxymethyl*-1 : 2-*benzanthracene (BB) and alpha-naphthalene acetic acid (ANAA) in S.G. broth*

Culture No.	Legend	Growth	Infl	F	Incubation	
					Days	Temp (°C)
I	0.0204 mg/ml BB	++++	++	++	5	20
II	0.0204 mg/ml BB	++++	++	++	5	30
III	0.1 mg/ml ANAA	++++	+	+	5	20
IV	0.1 mg/ml ANAA	++++	+	+	5	30
V	S.G. broth (control)	++++	+	+	5	20
VI	S.G. broth (control)	++++	±	±	5	30
VII	1 ml I, 0.0204 mg/ml BB	++++	++	++	5	20
VIII	1 ml II, 0.0204 mg/ml BB	++++	+	+	5	30
IX	1 ml III, 0.1 mg/ml ANAA	++++	++	++	5	20
X	1 ml IV, 0.1 mg/ml ANAA	++++	++	++	5	30
XI	1 ml V (control)	++++	+	+	5	20
XII	1 ml VI (control)	++++	+	+	5	30
I		++++	+++	+++	10	20
II		++++	+++	+++	10	30
III		++++	+++	+++	10	20
IV		++++	+	++	10	30
V		++++	+	++	10	20
VI		++++	±	+	10	30

BB and ANAA were suspended in 30 ml amounts of S.G. broth contained in 125 ml erlenmeyer flasks, with resultant concentrations of 0.0204 mg/ml and 0.1 mg/ml, respectively. These flasks were prepared in duplicate so that they could be incubated at both 20° and 30° C. All flasks, plus 2 controls, were inoculated with 0,1 ml amounts of a washed cell suspension of

S. cerevisiae. After 5 days of incubation the cultures were examined for Y → F activity, and a 1 ml aliquot from each was transferred to a flask containing media of the same composition as that from which the 1 ml aliquot was taken. These were likewise incubated for 5 days and also the original flasks were incubated.

Table 23 contains the data for this experiment. It can be seen that the Y → F activity found in the flasks that received the 1 ml aliquot, after 5 days of incubation was no greater than that found in the first set of flasks after the first 5-day period of incubation, whereas the first set incubated for 10 days showed an increase in Y → F activity over that found after 5 days. If the Infl and F forms were genetically stable, a greater proportion of them, as compared to their incidence in a yeast phase culture, would have been carried over in the 1 ml aliquot and they should have predominated after growth in the presence of the supposedly selective agent was permitted. This did not occur.

Discussion and Summary

Certain of the chemicals tested in this study, e.g., BB, exhibited stronger Y → F activity in S.G. broth cultures, both when incubated with shaking and in stationary cultures, than in the synthetic medium. In the S.G. broth, cultures incubated with shaking showed superior Y → F activity (tables 3, 4, and 6).

It is pertinent to this phenomenon to record the work of SELZER and BAUMBERGER (1942), who showed that the endogenous respiration of *S. cerevisiae* is not influenced by metallic mercury in the suspending medium for the cells, but that the exogenous respiration system is markedly inhibited. They suggested that the sulfhydryl groups, which are specifically inhibited by mercury, of the endogenous respiration systems are located within the interior of the cell, while the -SH groups concerned in the exogenous respiration system are located in or near the cell wall. BERNHEIM (1942) similarly found that the exogenous respiration of *B. dermatitidis* was sensitive to M/100 sodium cyanide, whereas the endogenous respiration was relatively insensitive. Evidence in support of the spacial separation of the endogenous and exogenous systems also comes from the works of STEIR and STANNARD (1936), WINZLER and BAUMBERGER (1938), and SPIEGELMAN and NOZAWA (1945).

The #20 synthetic medium contains no -SH groups, whereas the S.G. broth doubtless does. It is possible that there is a greater degree of endogenous respiation of the yeast cells occurring in the synthetic medium than in the S.G. broth. Whether this difference could be attributed to differences in oxygen tension among S.G. broth cultures incubated with shaking, S.G. broth stationary cultures, and #20 medium stationary cultures is not known.

The work of O'CONNOR (1950b) has shown that fluoride and iodoacetate, because of their action on -SH groups, inhibit aerobic glycolysis in embryonic chick midbrain tissue and that such an inhibition is accompanied by a decrease in the rate of cell division. This association between cell division and aerobic glycolysis is in accord with the association found in normal chick embryo development (O'CONNOR, 1950a). Conclusions from O'CONNOR's work may not be properly applied here, since no comparative metabolic data exist for yeast growing in S.G. broth and in #20 medium. Nevertheless it would be expected that cells undergoing limited aerobic glycolysis and/or an increased endogenous respiration might be less susceptible to the effects of agents whose action is on -SH containing groups and thence on the cell division mechanism. Support for the postulation that cells of *S. cerecisiae* may have a greater endogenous respiration in #20 medium than in S.G. broth as yet lacks experimental confirmation.

The inhibitory action of iodoacetate on cell division of the midbrain of 6-day-old chick embryos may also be correlated with its ability to combine with -SH groups (O'CONNOR, 1950b). The favorable results obtained with iodide therapy in certain cases of systemic mycotic infections (MARTIN and SMITH, 1939a, 1939b) might profitably be re-investigated with the idea of ascertaining the effect of iodides on the cell-division mechanism of yeasts.

The significance of -SH groups for the cell division mechanism appears, in general, to be borne out on observations with plant and animal cells. The study made by GREGORY and CASTLE (1931) on the development of rabbit eggs implicates -SH groups in the cell division and general growth rate processes. After 40 hours of development, eggs from large size races of rabbits produced a larger number of cells in the process of division than did eggs of small size races. The suggestion was made, based on HAMMETT's (1930) postulate that the "-SH group is the essential chemical stimulus to growth by increase in cell number", that a greater amount of -SH groups in the nucleus may be responsible for the more rapid growth rate of eggs and embryos from large-race matings.

Further evidence for the -SH nature of the enzyme(s) which regulate cell multiplication can be gleaned from the work of THIMANN (1949). The action of a number of -SH inhibitors was studied, using the effect on the amount of curvature and growth of slit stems of peas as a test method. Arsenite and the organic arsenical mapharsen inhibited growth strongly, as did iodoacetate and parachloromercuribenzoate. The last compound is a more specific -SH reagent than the others. It was first used by HELLERMAN, CHINARD, and DIETZ (1943). At concentrations which produced 50 % inhibition of growth, arsenite, ioacetate, and parachloromercuribenzoate did not exert a detectable effect on the coleoptile respiration. RYAN, TATUM, and GIESE (1944) showed that the growth of *Neurospora* is inhibited by iodoacetate in a manner both quantitatively and qualitatively similar to that in the coleoptile sections in the work of THIMANN (1949). The observations of THIMANN, that the inhibition of growth by -SH inhibitors occurs in the presence of auxin, appears contradictory to the concept propounded here that -SH inactivation may lead to auxin production in *S. cerevisiae.* There is no doubt that there are many -SH activated enzymes in a living system and that they probably could be graded as to their relative susceptibility to -SH inactivating agents. It would be expected that physiological damage would increase progressively with an increase in the concentration of the agents being used. It is pertinent to note here that THIMANN and BONNER (1949) were able to inhibit the growth processes of Avena coleoptiles elongating in auxin and sucrose, and slit internodes of Pisum stems curving in auxin alone, with the lactones coumarin and protoanemonin. That both of these compounds act by reacting with a SH-containing enzyme was supported by the fact that BAL prevented the growth inhibition caused by these compounds. However, at sub-inhibiting concentrations the growth of the test plants was *promoted* in each case. This observation lends support to the auxin concept for Y → F formation, since it was shown by THIMANN and BONNER that more auxin was produced, at least as evidenced by the Avena and Pisum tests, in the presence of sub-inhibiting concentrations of the -SH inhibiting lactones, coumarin and protoanemonin.

The work reported here lends only little support to the idea that the inactivation of SH-containing compounds resulted in the formation of Infl and F forms of *S. cerevisiae.* The fact that BB plus cysteine gave greater

Y → F activity than BB plus cystine or serine (tables 4, 5b, 9 and 11) suggests that — SH groups were involved, because the reduced S group is the only component of the cysteine molecule not shared by serine and cystine.

One of the difficulties in elucidating the mechanism responsible for dimorphism in microorganisms is that it is apparently so labile that many agents of divergent chemical and physical activity are capable of interfering with it. In addition to the agents already mentioned, Y → F and B → F activity have been attributed to x-rays (EISENSTARK and CLARK, 1947), gamma rays (LEA, HAINES, and COULSON, 1937), radium (SPENCER, 1935), magnesium ions (WEBB, 1949a, 1949b), and many other agents. The many compounds of divergent chemical structure which induce the cf-reaction have already been mentioned (LEVAN, 1947).

Most of the features of the cf-reaction indicated previously are also characteristic for the Y → F activity. LEVAN has had the opportunity to examine copies of fig. 6 and 21 and is of the opinion (personal communication) that both photographs represent instances of the cf-reaction, although fig. 6 may represent a more regular (less narcotized) growth than fig. 21.

The compounds which have been found to enhance the Y → F, Y → M, and B → F formation and the cf-effect in microorganisms, may have that characteristic in common which concerns their mode of detoxification when cells are exposed to them. Cysteine is one of the few amino acids which may be used by an organism for detoxification (FIESER and FIESER, 1944). FIESER and FIESER point out that, at least for higher animals, the chloro, bromo, and iodo derivatives of benzene are all excreted as derivatives of mercapturic acid in which the amino group of the cysteine residue is acetylated. Naphthalene and anthracene are detoxified by some animals in the same way.

Some evidence from the work reported here, supplemented by the results of other investigators, does, in some measure, support the contention that auxins may be responsible for the elongation of cells of *S. cerevisiae.* Significant points may be summarized as follows:

1. The auxin activity of filtrates from cultures containing numerous filamentous cells of *S. cerevisiae* was higher than that found in filtrates from cultures that were predominantly in the yeast phase.
2. Tryptophane (hydrochloride), which is a precursor of indole acetic acid, induced the formation of a relatively large number of filamentous cells in #20 synthetic medium.
3. Increasing the hydrogen ion concentration of cultures containing tryptophane enhanced the Y → F phenomenon considerably, presumably by releasing the free acid form of heteroauxin from the inactive complex.
4. The effect of ANAA on the growth of *S. cerevisiae* appeared to be that of increasing the number of F forms without affecting the rate of cell multiplication; a comparable phenomenon occurs in higher plant cells exposed to auxins.

If auxin induces elongated cells in yeasts, then this mechanism might also explain filament (B → F) formation in bacteria. The smooth to rough (S → R) or Y → F variation in yeasts (PUNKARI and HENRICI, 1933) and yeastlike fungi (MICKLE and JONES, 1940) shows such a close parallelism to this type of variation in bacteria that it is very likely that the mechanism governing S → R, Y → M, Y → F, and B → F formations are the same. LIESKE (1921) suggested that unicellular bacteria bear a relation to the actinomycetes similar to that of the yeasts to molds. NOVAK and HENRICI (1933) were able to recover a yellow staphylococcus from Berkefeld-filtered cultures of an actinomycete after incubation for 5 weeks at room temperature. This "staphylococcus" was morphologically and culturally indistinguishable from other staphylococcus strains grown on ordinary media. On glucose agar the coccoid strain grew in the form of rods and branching filaments, but these reverted to cocci when transferred again to plain agar.

The growth of bacteria has been associated with 3-indole acetic acid for some time (SALKOWSKI and SALKOWSKI, 1880; SALKOWSKI, 1885a, 1885b). *Proteus vulgaris* has been found to produce 3-indole acetic acid (HERTER and TEN BROEK, 1909). HERTER (1908) demonstrated that the 3-indole acetic acid found in the urine of patients came from bacteria that were active in the putrefaction of intestinal contents. The demonstration that typhoid bacilli in the urine of urinary carriers are frequently in the form of long filaments (JORDON and BURROWS, 1945) may well be correlated with the very high heteroauxin content of human urine (KOGL, HAAGEN-SMIT, and ERXLEBEN, 1933).

A study of CHOLODNY and BELTYUKOVA (1939) on the effect of phytohormones on a number of microorganisms, among which were *Bacillus subtilis, Bacillus mycoides,* and *Saccharomyces cerevisiae,* showed that often an abnormal enlargment of the cells was produced.

Thus it would appear that the induction of filament formation in bacteria is probably of the same nature as the mechanism responsible for Y → F formation in yeast cells.

One of the ramifications of this work concerns the genetics of Y → F formation: the question as to the inheritance of the factor of resistance or susceptibility of yeasts to agents inducing dimorphism; the relative effects on haploid and diploid yeast cultures of Y → F inducing agents; the genetic factors determining the stability or instability of F forms when they are removed from the Y → F enviornment. The work of LINDEGREN (1944) indicates that the genetic constitution of the cell may be of fundamental significance in Y → F effects. Haploid cultures examined by LINDEGREN showed many extermely thin elongated cells, which are generally absent from diploid cultures. Also, haploid cells tended to grow in clusters because of failure of the full-sized daughter cells to separate. Such clusters sometimes were large enough to settle to the bottom of the tube and they contained long, thin cells resembling a mycelium. Comparable observations have been made on numerous occasions during the course of this work, yet diplophase cultures were employed here exclusively, as indicated by the morphology of cells and colonies and the observation of ascospore formation within F forms.

The failure to precipitate a deep infection, under conditions conducive to F → Y formation (bodily temperature of approximately 37° C), with dermatophytes (F phase) that do not exhibit dimorphism has already been mentioned (SULZBERGER, 1929; BROCQ-ROUSSEU *et al.*, 1926). It may be that Y and F formation results from biochemical pathways that are mutually exclusive and that the transition from one phase to the other is governed by rather restricted nutritional and temperature requirements.

An attempt toward applying the auxin concept for Y → M formation *in vivo* by treating mice systemically infected with *Candida albicans* with compounds having plant growth activity will be reported in a subsequent paper.

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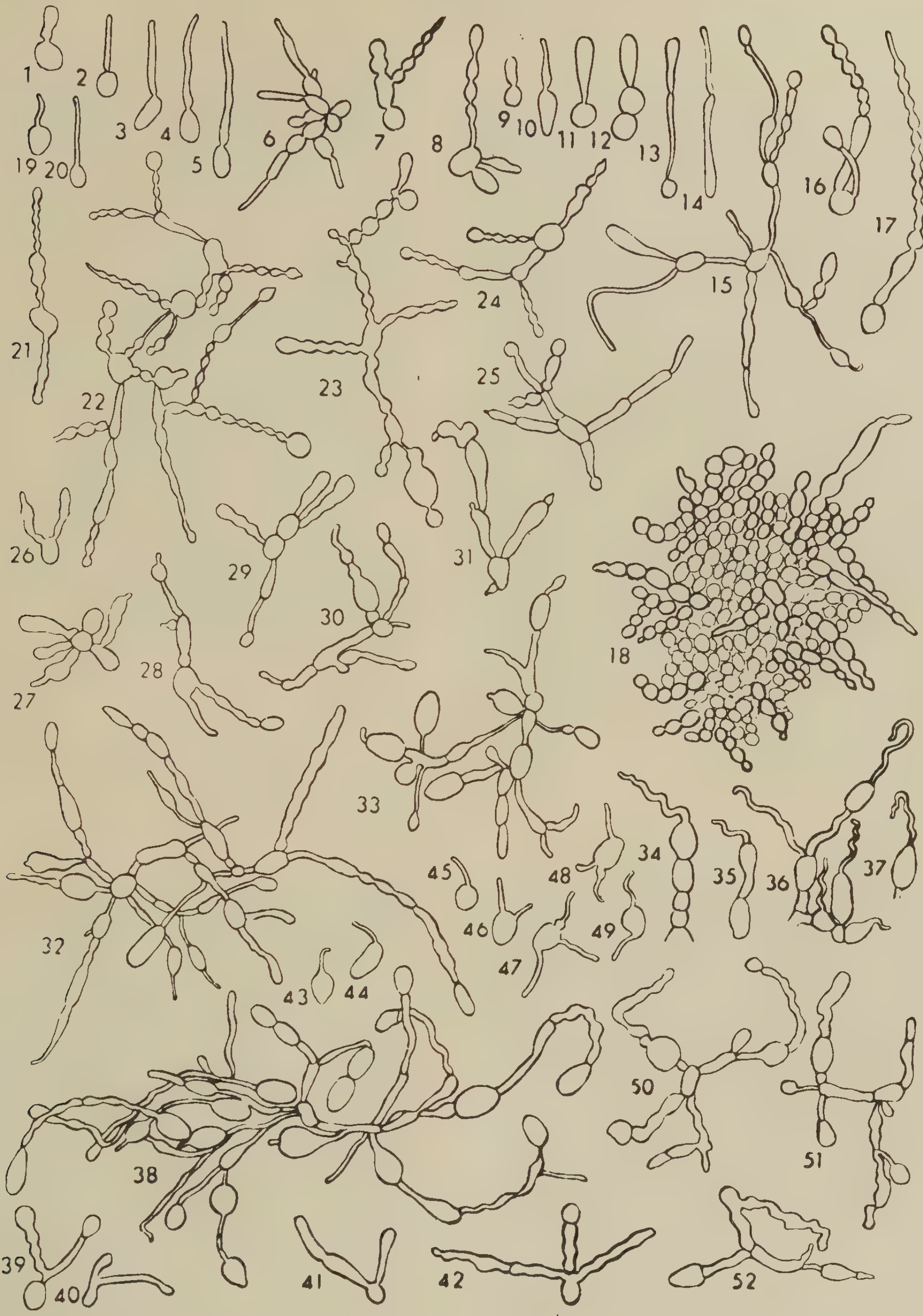
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Fig. 1. Outline drawings of cf-forms induced by aliphatic alcohols: methanol (1—14), ethanol (15—18), propanol (19—25), normal butanol (26—37), iso-butanol (38), tertiary butanol (39—42), iso-pentanol (43—52). 400×. Reproduced from LEVAN (1947).

Courtesy Prof. ALBERT LEVAN.

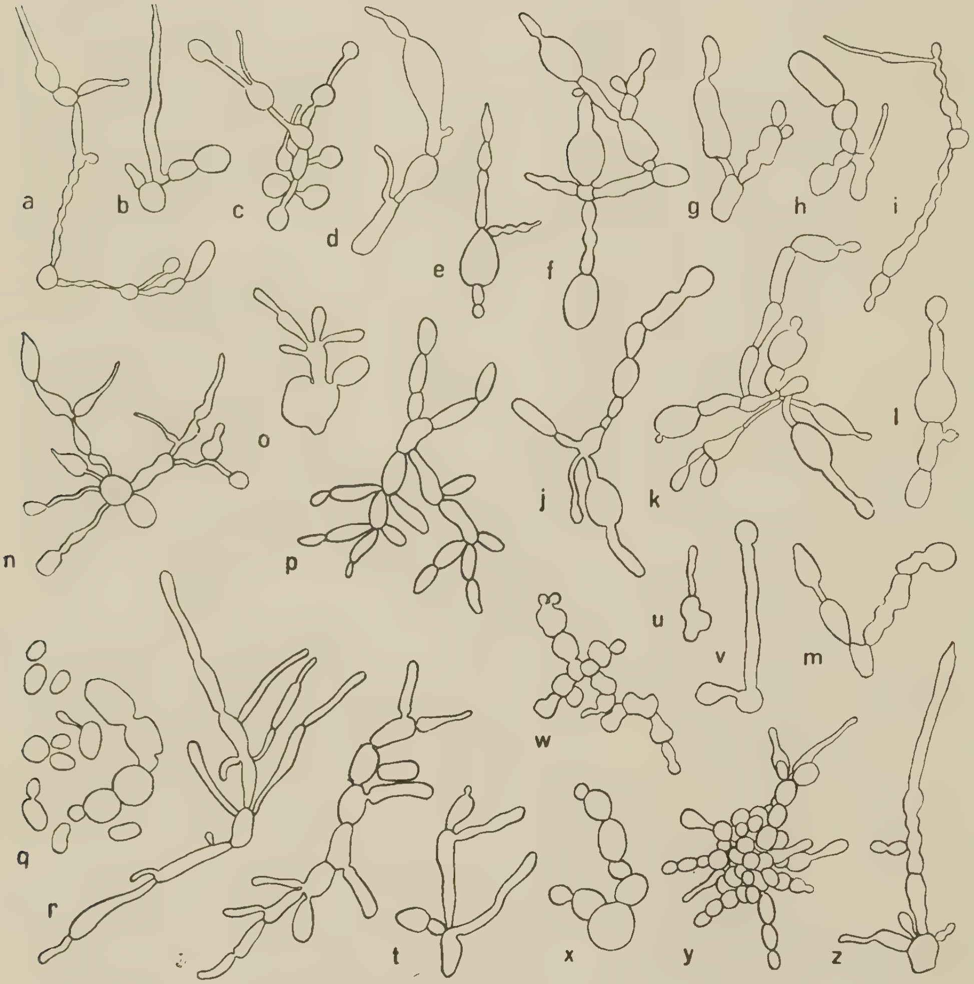
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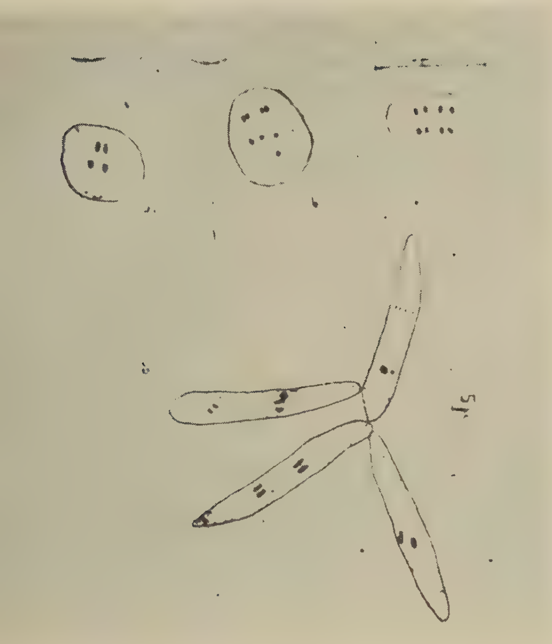
Fig. 2. Outline drawings of cf-forms induced by various aliphatic substances: ethyl ether (a—f), acetone (g—n), chloroform (o, p), paraldehyde (q, w), chloral hydrate (r—t,) urethane (u—v, x—z). 500×. Reproduced from LEVAN (1947).

Courtesy Prof. ALBERT LEVAN.



ig. 3. *S. cerevisiae* exposed to acenaphthene in wort edium for 6 hr. resulted in filamentous cells. The elongated cells and some of the yeast phase cells (5) contain chromosomes, according to SUBRAMANIAM. Reproduced rom SUBRAMANIAM. (1945) Courtesy Current Science Assoc., Bangalore, India

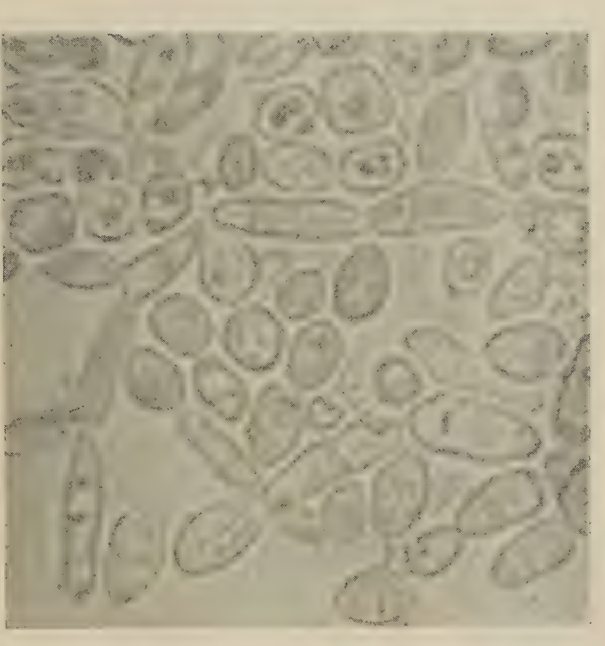


Fig. 4. *S. cerevisiae* exposed to 0.204 mg/ml of 9 : 10-bishydroxymethyl—1 : 2—benzanthracene (BB) in S.G. broth for 4 weeks at room temp. Elongated cells occur in the form of filaments. 970×.

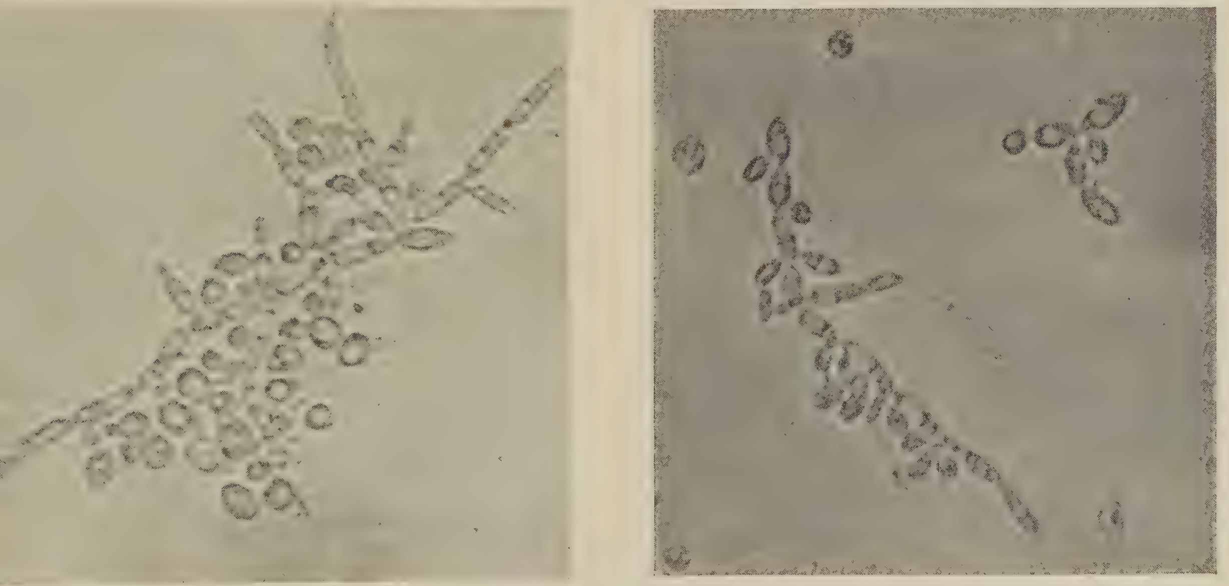


Fig. 5. *S. cerevisiae* exposed to 0.29 mg/ml of BB in S.G. broth for 15 days at room temp. Numerous yeast phase cells are still evident. 440×.

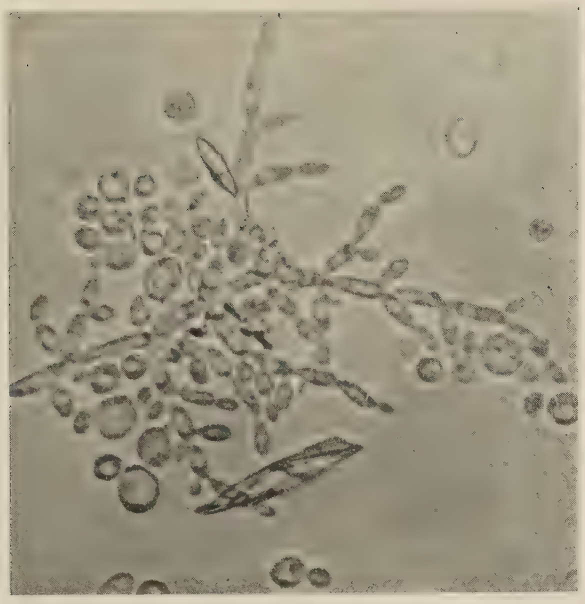


Fig. 6. *S. cerevisiae* exposed to 0.29 mg/ml of BB in S.G. broth for 21 days at room temp. Yeast phase cells are still evident and crystals of BB are also visible. 440×.

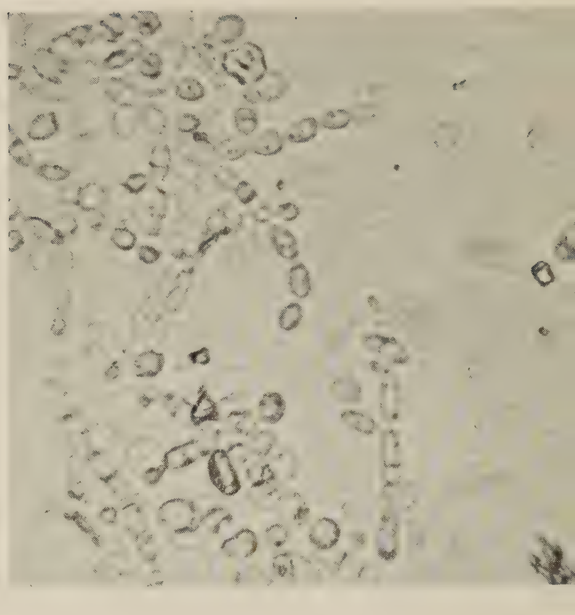


Fig. 7. Same conditions as in fig. 6. Note the strings c yeast cells which have not elongated. 440×.

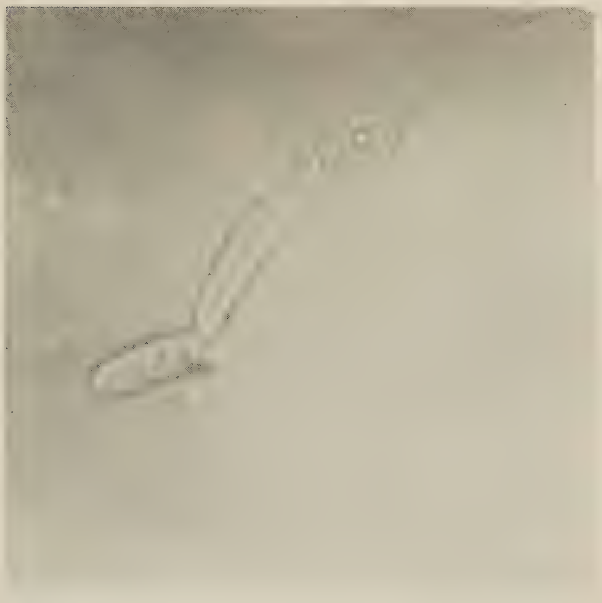


Fig. 8. *S. cerevisiae* exposed to 0.29 mg/ml of BB in S.G. broth for 26 days at room temp. Note the racquet mycelium. 970×.

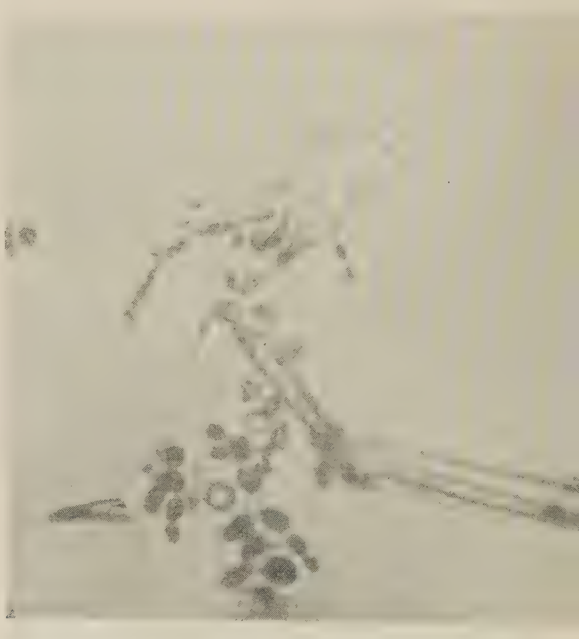


Fig. 9. *S. cerevisiae* exposed to 0.29 mg/ml of BB in S.G broth for 26 days at room temp. Note the many cells tha were unaffected morphologically. 440×.

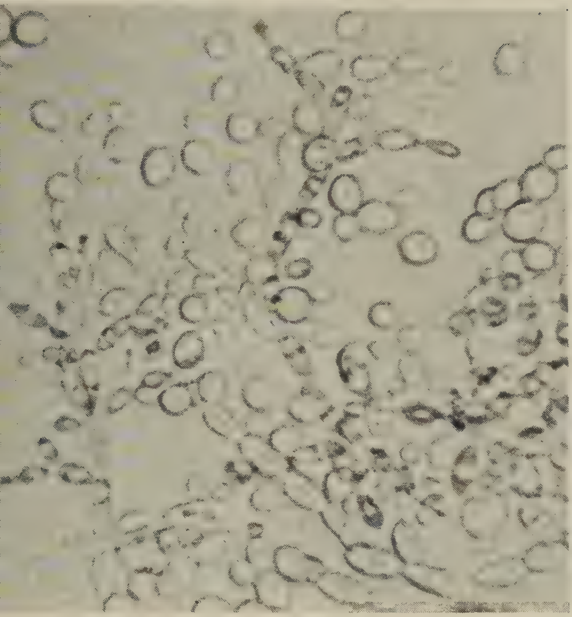


Fig. 10. *S. cerevisiae* exposed to 0.29 mg/ml of BB in .G. medium for 37 days at room temp. Yeast phase cells re still evident, filaments of elongated cells are very long and occur in packets. 440×.

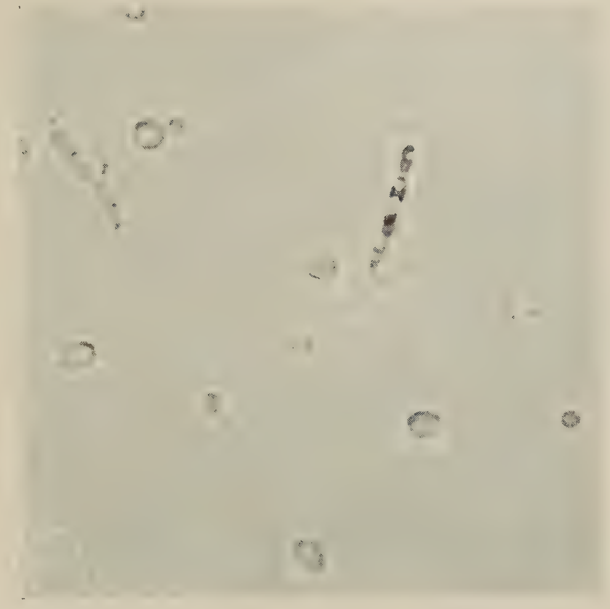
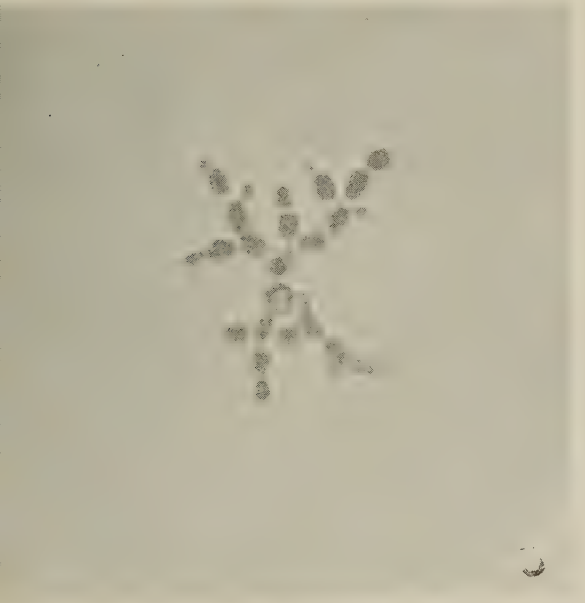


Fig. 11. *S. cerevisiae* exposed to 0.0204 mg/ml of BB in S.G. broth for 99 hr. at room temp. 440×.



ig. 12. *S. cerevisiae* exposed to 0.0204 mg/ml of BB in .G. broth for 10 hr. at room temp. This fig. demonstrates essation of cell division without occurrence of cell elongation. Stained with cotton blue. 440×.

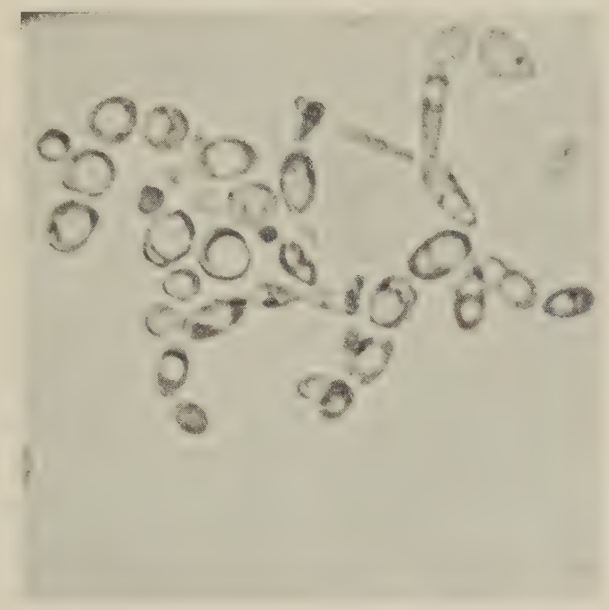


Fig. 13. *S. cerevisiae* exposed to 0.0204 mg/ml of BB in S.G. broth culture incubated with shaking for 99 hr. at room temp. 440×.

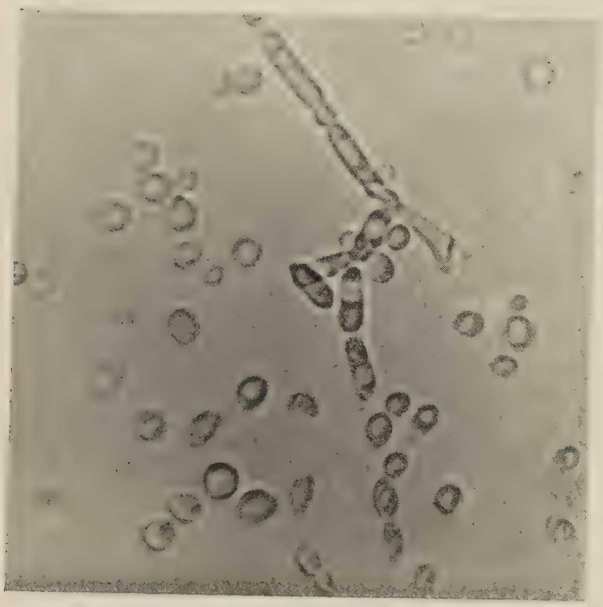


Fig. 14. *S. cerevisiae* exposed to 0.0204 mg/ml of BB in S.G. broth culture incubated with shaking for 99 hr. at room temp. 440×.

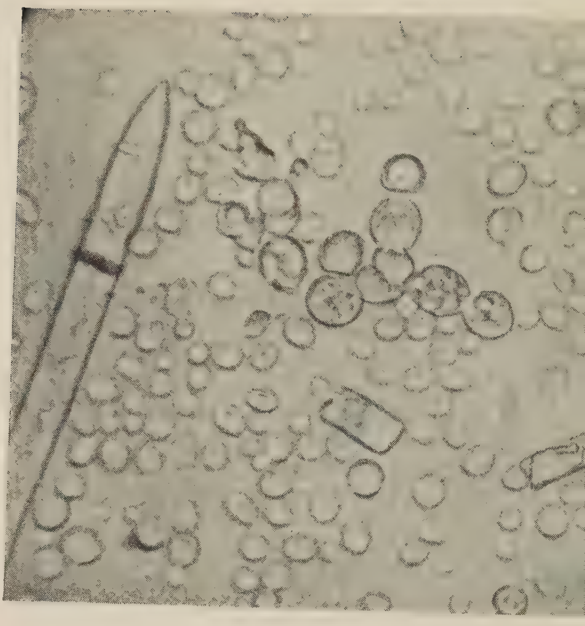


Fig. 15. *S. cerevisiae* exposed to 0.00002 mg/ml of B in S.G. broth culture incubated for 195 hr. at room temp with shaking. Note giant yeast cells and crystals of BB 440×.

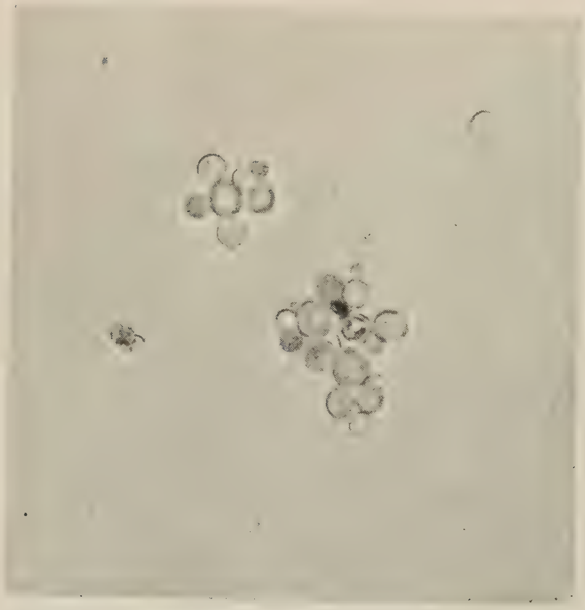


Fig. 16. *S. cerevisiae* exposed to 0.0204 mg/ml of BB plus 0.01 molar l-cysteine in #20 synthetic medium for 5 days. 440×.

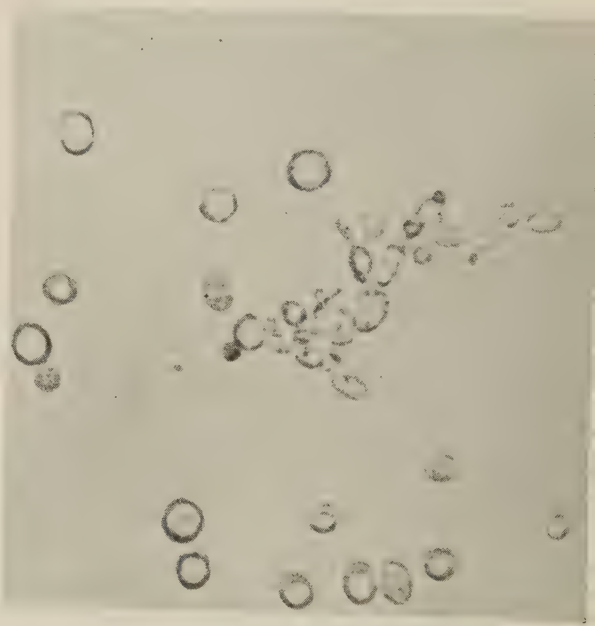


Fig. 17. *S. cerevisiae* in S.G. broth exposed to 0.01 mg/ml of ANAA after 2 days incubation at room temp. Note that the Infl contains yeastlike and elongated cells. 440×.

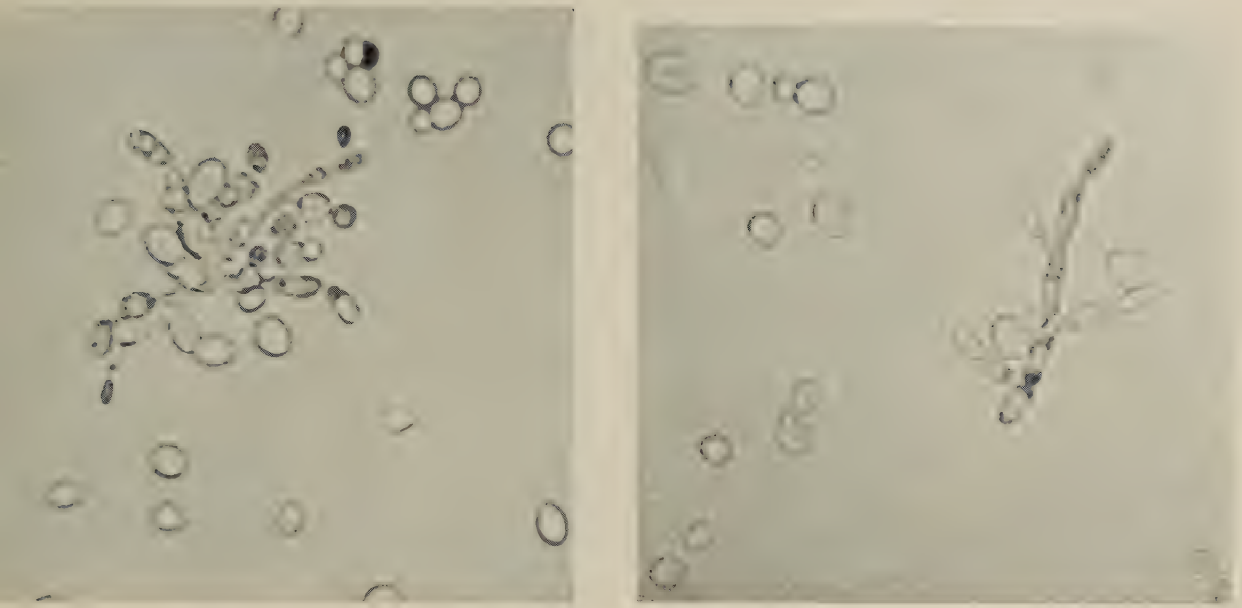
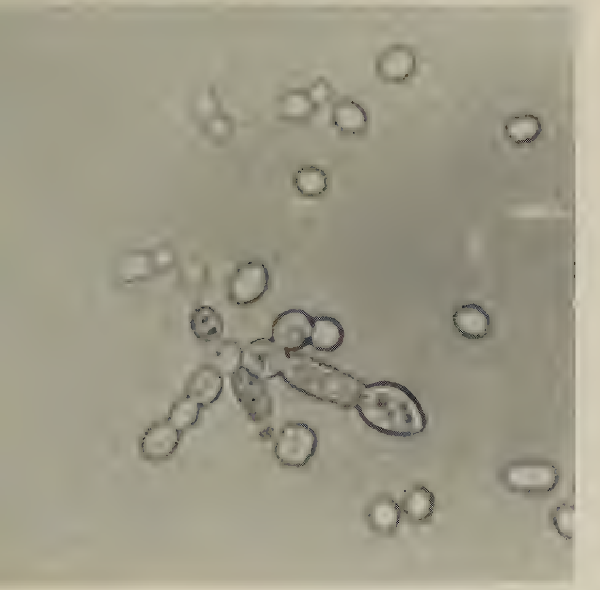


Fig. 18. *S. cerevisiae* in S.G. broth exposed to 1.0 mg/ml of ANAA for 4 days at room temp. 440×.



ig. 19. *S. cerevisiae* in S.G. broth exposed to 1.0 mg/ml f ANAA for 4 days at room temp. The longest filament may be multinucleate. 440×.

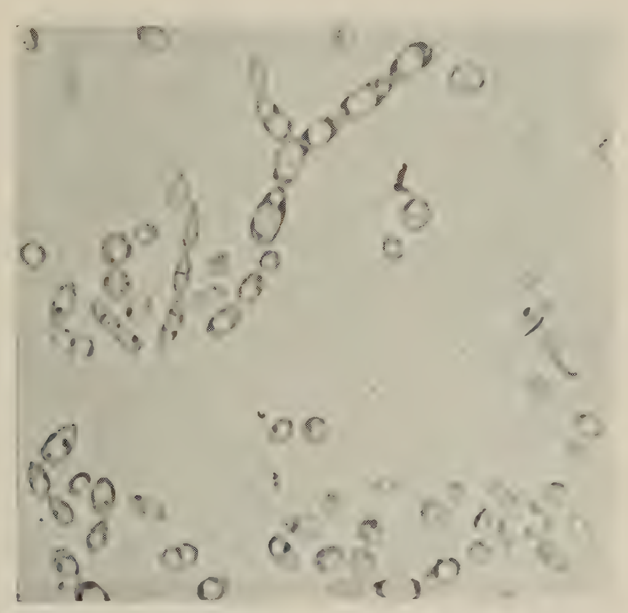


Fig. 20. *S. cerevisiae* in S.G. broth exposed to 0.1 mg/ml of ANAA for 4 days at room temp. 440×.

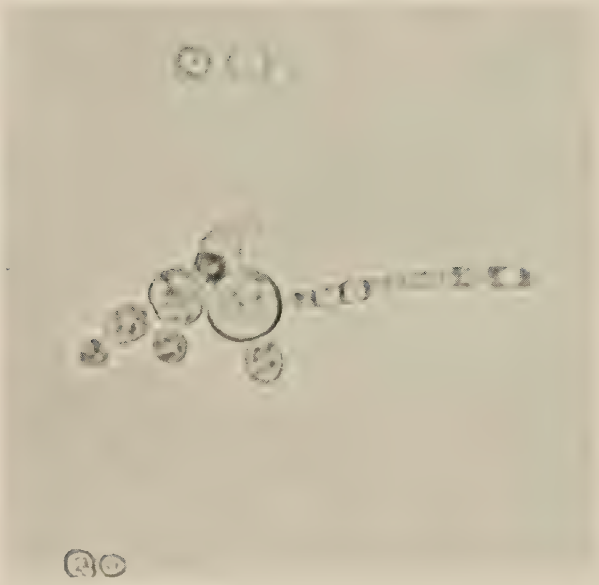


Fig. 21. *S. cerevisiae* exposed to 0.01 molar l-tryptophane HCl in #20 synthetic medium after 10 days at 20° C. 440×.

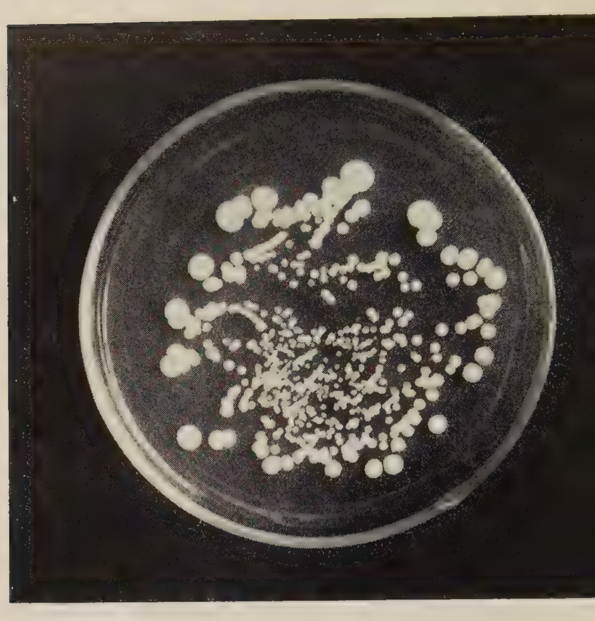


Fig. 22. *S. cerevisiae* on S.G. agar incubated at 20° C for 8 days.

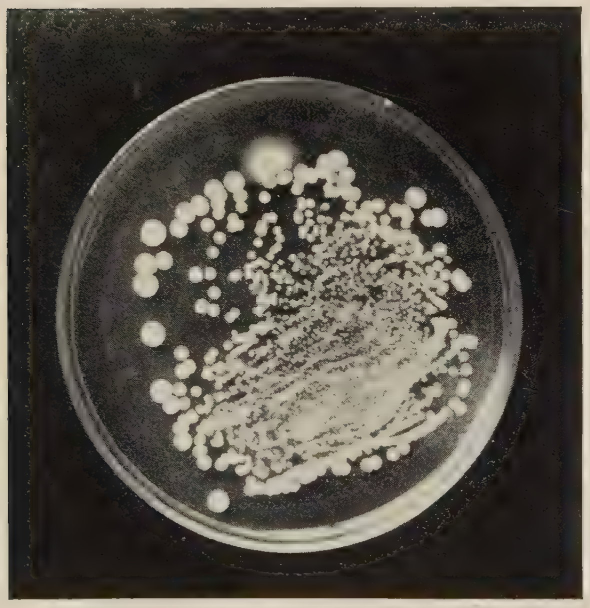


Fig. 23. *S. cerevisiae* on S.G. agar incubated at 30° C. for 8 days.

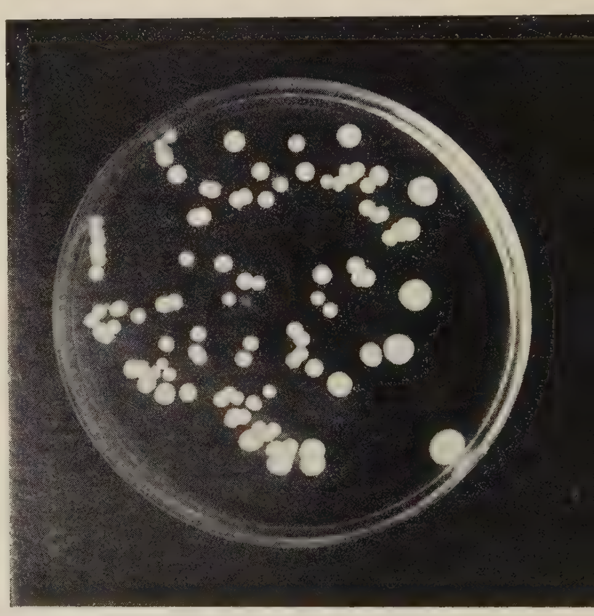


Fig. 24. *S. cerevisiae* on S.G. agar incubated at 37° C for 8 days.

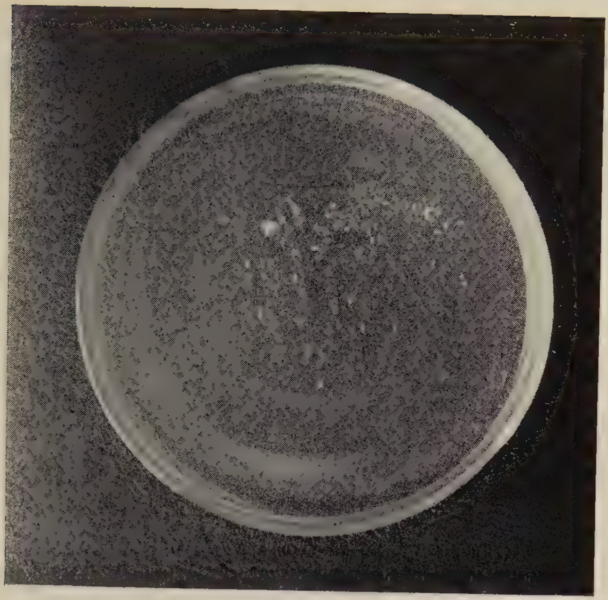


Fig. 25. *S. cerevisiae* on S.G. agar containing 1 mg/ml of ANAA, incubated at 20° C for 8 days.

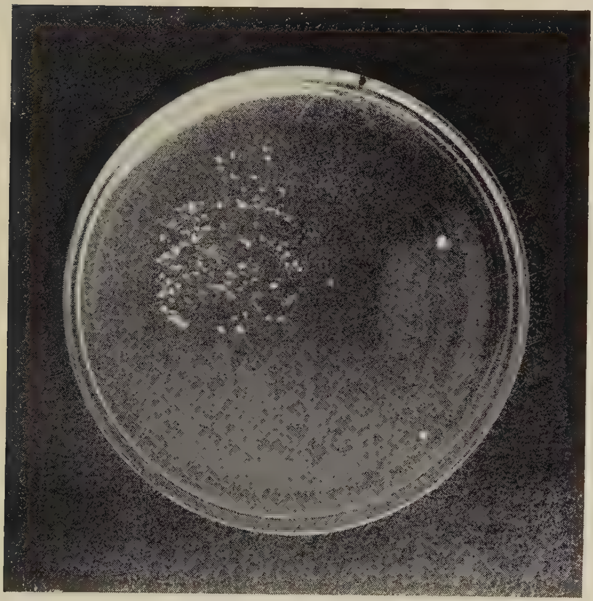


Fig. 26. *S. cerevisiae* on S.G. agar containing 1 mg/ml of ANAA, incubated at 30° C for 8 days.

Cryptococcus neoformans Strains from a Severe Outbreak of Bovine Mastitis.

by

CHESTER W. EMMONS

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(30.1.1952)

POUNDEN, AMBERSON and JAEGER (13) and INNES, SEIBOLD, and ARENTZEN (7) have studied an unusual and severe outbreak of bovine mastitis caused by *Cryptococcus neoformans* (*Torula histolytica*). The outbreak had occurred in a herd of about 235 head and involved 106 animals. Routine methods of control had failed to check the outbreak and in November 1950, Dr. POUNDEN was asked to investigate it. He recognized that an unusual type of mastitis was present in the herd and made extensive laboratory examinations of quarter milk samples from the producing cows for detection of previously unrecognized cases and of the abnormal secretions from severe cases of mastitis.

The etiologic agent was isolated from milk samples in Dr. POUNDEN's laboratory and from a biopsy of udder tissue in Dr. SEIBOLD's laboratory and was received from them by the writer for identification. Its apparent identity with the species *C. neoformans* indicated that in addition to the economic loss facing the dairy, the men handling the herd and the milk were exposed to a grave potential danger. The situation therefore posed a considerable public health problem. This has stimulated extensive studies of the outbreak, including comparison of strains isolated from the cattle with strains from human sources and from soil, and attempts to isolate the fungus from the environment of the dairy herd.

Although this outbreak appears to be unique among reported outbreaks of bovine mastitis there are four published reports which indicate that *C. neoformans* may occasionally infect cattle. KLEIN (9) in 1901 isolated a "yeast" from a guinea pig inoculated with a pooled milk sample in a routine search for tubercle bacilli. From his description it is probable that the "yeast" was *C. neoformans*. CARTER and YOUNG (2) recently reported a similar isolation and identified the organism as *C. neoformans*. SANFELICE (14) isolated an organism from a tumor in an ox which he stated was like *C. neoformans* except that it induced deposition of calcium in experimentally infected animals. However, COX and TOLHURST (3) observed deposition of calcium in animals infected with all their strains of *C. neoformans*, so that this apparently is not a specific difference, and Sanfelice's strain can be identified as *C. neoformans*. MADSEN (11) isolated an organism which he identified as *C. rotundatum* from purulent lymph nodes of a cow. It was pathogenic for guinea pigs and therefore probably was *C. neoformans*. The differential points he mentions are probably not significant. Mastitis was not reported in any of these cases nor have we found a report in which *C. neoformans* was incriminated in mastitis.

Among the problems presented by this interesting occurrence of *Cryptococcus mastitis* in dairy cattle, this paper is concerned primarily with the identity of the fungus, a comparison with strains from human sources as to virulence and other characteristics, attempts to isolate the fungus from sources other than milk and tissues and possible sources of infection in the environment of the cattle.

Environmental Studies:

In human cryptococcosis of the central nervous system it is believed that in many cases the primary lesion is in the lungs, although the frequency with which this occurs is unknown. In the present outbreak of mastitis the principal lesions involved the udder and regional lymph nodes and the disease appeared to develop as a retrograde infection via the milk ducts but evidence of systemic infection was found by examination of organs and tissues at autopsy in one animal (7). Cuds and feces were examined in an attempt to determine whether ingested food was contaminated by Cryptococcus. It was assumed that obvious gross contamination of floors and lots by milk which contained enormous numbers of the yeast cells gave opportunity for infection or reinfection of the respiratory and gastrointestinal systems if the cow is susceptible to such infection.

Eleven fecal specimens from cows with mastitis were examined by direct culture on neopeptone-dextrose agar with penicillin and streptomycin added and were injected intraperitoneally into mice by a technic which has been found useful in this laboratory for the isolation of pathogenic fungi from contaminated sources (4,5). Yeasts, Candida, Mucor and other fungi were readily isolated in large numbers by direct culture but *C. neoformans* was not isolated either directly or by passage through mice. Five cuds were similarly examined without obtaining the pathogen. In subsequent examination of feces from a calf experimentally fed large doses of living *C. neoformans* (13) we were unable to recover the fungus even though fecal specimens were taken over a period of seven days following the infectious feedings.

Highly viscid, mucoid milk, which in some instances was essentially a culture of *C. neoformans* was observed dripping from the teats of cows with mastitis and this natural seeding of the floor would seem to assure a heavy contamination of the barn in which cows with mastitis were kept. Success in isolating this and other pathogenic fungi from soil (4,5) offered a proved method for use, but in spite of these apparently favorable factors, attempts to isolate *C. neoformans* from litter in the barn where infected cows were isolated were not successful. Specimens were not taken from directly under the cows where contamination presumably was heaviest, but 22 specimens of litter from near the feeding troughs, dust from the stanchions, and various types of feed were examined by direct culture and by passage through mice. In addition 48 agar plates were exposed in the alley back of the milking stanchions while the cows were in the barn. Although yeasts, Cryptococcus-like fungi, Mucor, Aspergillus, Penicillium and other fungi were readily isolated in these attempts, the pathogen was not found. The fungicidal effectiveness of cleaning methods used in the barns and of the natural air-drying of the fungi have not been properly investigated, but we did not obtain any direct evidence that there was a high level of contamination by viable *Cryptococcus neoformans* in the barn.

Study of Strains:

In the present study the original strain isolated by Pounden and Seibold, 26 strains isolated by Pounden from quarter milk samples, and 7 strains isolated by the writer from milk samples or tissues obtained at autopsy were compared with strains from human cryptococcosis (torulosis), the original strain of this fungus isolated by SANFELICE in 1894 and strains isolated from soil (5). The 62 strains which were isolated by POUNDEN over a comparatively short period of time from quarter samples of milk were taken as representative and were most carefully studied. They were grown upon neopeptone-dextrose and cornmeal agars for comparative studies of cell morphology and upon 7 carbohydrates known to be critical for this fungus. The strains exhibited the spherical, budding, encapsulated cells characteristic of the species.

None of the strains fermented sugars but acid was formed slowly and in small amounts, sometimes only in the gas tube of Dunham fermentation tubes, in certain sugars. All 62 strains produced acid in glucose, levulose and mannose; 50 produced acid in sucrose; 24 in maltose; 19 in starch; and none in galactose. In their erratic action upon carbohydrates these strains resemble strains of the species isolated from human sources and from soil when tested under similar conditions (3, 5, 6).

These strains grew readily at 37° C. This aids in differentiation of *C. neoformans* from *C. rotundatum* and some other encapsulated yeast-like fungi which grow poorly or not at all at this temperature.

The first strain isolated was tested for heat resistance. It was killed by 5 minutes exposure to a temperature of 55° C.

Despite the single epidemic source of these strains there was considerable variation in gross characteristics as well as in physiological activity. This also is in accord with observations of strains from different human sources (1, 3, 8, 10, 12). The most easily observed differences were in color, which varied from cream to deep tan, and in the mucoid consistency of colonies which varied from a firm pasty colony to one which was so mucoid that it flowed to the base of a slant.

Of the 62 strains systematically compared as to these characteristics 15 were arbitrarily classified as "dry tan", 27 as "intermediate tan", 11 as "intermediate cream", 1 as "mucoid tan", 6 as "mucoid cream" and 2 as mixed types when first tested. The formation of sectors and of secondary colonies in old cultures indicated that these variations arose as a result of the inherent instability of the organism. The origin of mucoid variants in 10 strains was observed during the course of the studies.

Virulence:

Although all characteristics of a yeast-like fungus must be taken into consideration before it is identified as *C. neoformans*, a most important criterion is its virulence for mice. A few relatively avirulent strains have been studied and reported, but most strains from human cryptococcosis kill mice readily and quickly when injected intracerebrally and more slowly and less regularly when injected intravenously or intraperitoneally. In comparative studies it has been observed in this laboratory that when species of yeast and Candida are injected into the white mouse intracerebrally in doses up to 10^7 cells per mouse the animal does not die of the infection and, with few exceptions, the fungus can not be recovered in culture from the brain if the animal is killed at the end of a month. On the other hand, when *C. neoformans* is injected intracerebrally at the level of 10^5 cells per mouse the animals usually die in from 5 days to 2 weeks. A smooth steep death curve does not always result, probably because the exact locus of inoculation and the amount retained within the cranium vary from animal to animal through variations in technic, but the method is practically useful. The mouse becomes lethargic, the coat is rough, and the cranium is raised to a marked degree due to internal pressure.

Forty mice were inoculated intracerebrally with strains from mastitis. The inoculating doses were varied, and a few of the animals survived to 26 days. Several mice were killed when clinically ill or moribund so that survival time was not determined for all. However, in one series in which 4 different strains from mastitis were compared, all of 16 mice given 10^5 cells per mouse died within 12 days. Upon examination of fresh wet smears of the brains of these mice large numbers of encapsulated cells could be observed, some of them with very wide capsules. A comparison of pasty with highly mucoid strains did not reveal a significant difference in virulence.

Summary

Cryptococcus neoformans was isolated from a severe outbreak of bovine mastitis. A comparison of 62 strains revealed considerable variation in color, acid production in sugars, and amount of capsular material. During the course of the study, the origin of 10 mucoid mutants was noted. The strains were compared with strains of the same species isolated from human cryptococcosis, from soil, and with the original SANFELICE strain. They are typical of the species *C. neoformans* in colony characteristics, morphology, temperature tolerance, acid production in sugars and pathogenicity for mice.

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Sporotrichosis with formation of sporotrichids

by

C. Jack Young, M.D., Paul O. Shackelford, M. D.,
John H. Lamb, M. D. and Rollin C. Koons, B. S.*

(4.2.1952)

Since sporotrichosis is endemic in the United States one must constantly be aware of its possible occurrence. The diagnosis of classical sporotrichosis, with an initial chancre on exposed areas and subsequent nodular lymphangitis, is almost infallible. However, diagnostic difficulties may be encountered when any deviation from this typical pattern occurs.

Lewis and Hopper [1]) describe seven clinical types of the disease: The localized lymphangitic type, disseminated subcutaneous type, disseminated, ulcerating type, the systemic cases, the epidermal type, verrucous types and allergic lesions of sporotrichids.

This case of localized sporotrichosis with secondary sporotrichid formation is reported because of its rarity and the diagnostic hurdles it presented.

Case Report

History: This 16-year-old white male first noticed the appearance of a 1.5 cm. soft, bluish, tender, non-ulcerating nodule on the right upper arm on September 1,1950. He thouhgt that he had some type of insect bite. However, he saw no ticks nor other insects in this area. His occupation was that of a farm lad. He indulged in the usual desire for sun-tanning and frequently went shirtless. He was seen by his family physician and received intensive penicillin therapy for 14 days. While receiving this therapy two similar satellite nodules appeared. On September 15, 1950 the patient was seen by a surgeon who made a tentative diagnosis of sarcoma and excised the lesions. The pathologist reported the specimen to be compatible with cutaneous tuberculosis. The operative wound did not heal and gradually enlarged. On November 20, 1950 the patient developed a generalized, non-pruritic, macular eruption. This eruption was not close enough to a course of penicillin therapy to warrant a diagnosis of a penicillid. The patient was seen by Dr. Fredk. H. Chard of Wichita, Kansas who diagnosed a deep fungus infection and successfully cultured a grayish-black colony which was not sufficiently characteristic for classification. We saw the patient through the courtesy of Dr. Chard. Past and family histories were noncontributory.

Physical examination: The patient was a normally developed youth. On the lateral aspect of the left upper arm was a 4 × 5 cm. purplish-red ulceration. The border was elevated and firm without undermining. The base was clean and granular. Diascopy revealed no "apple jelly" nodules. No lymphadenopathy was noted. Distributed over the trunk and extremities was a generalized, brownish, non-scaly, macular eruption which faded upon pressure. The remaining physical findings were normal.

Laboratory evalulation: Urinalysis was normal. Hemogram revealed a red blood count of 4.4 million and a white blood count of 5,800 with a differential of 61 neutrophils (1 stab), 36 lymphocytes and 3 monocytes. Weil-Felix agglutinations and blastomyces complement-fixation tests were negative [1]). Tuberculin and histoplasmin skin tests were negative.

Biopsy specimen: Presented pseudoepitheliomatous hyperplasia with a dense infiltrate of mature lymphocytes. The infiltration was compatible with lymphoblastoma except for the maturity of the lymphocytes. No spores or hyphae were seen.

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*) Duke Laboratories, Stamford, Connecticut.

Mycology: Fifteen-day cultures on Sabouraud's medium revealed a black, filamentous colony which did not invade nor colorize the medium. Culture mounts presented branching hyphae with atypical pyriform buds. A similar, nonpigmented, yeast-like colony developed on blood agar base. A suspension from these colonies was injected intraperitoneally into a white rat. By the seventh day, when the animal was sacrificed, the testes were 4 times enlarged and quite tender. Tissue imprints from the testicles revealed gram-positive, cigar-shaped, intracellular organisms. Cultures of testicular tissue on Sabouraud's medium developed a jet-black colony with numerous central convolutions. Culture mount presented hyphae with pear-shaped terminal conidia in triads and tetrads. These findings were considered characteristic of Sporotrichum schenckii.

Clinical course: The patient was given a saturated solution of potassium iodide, 20 drops orally, 3 times a day. After a week of therapy he developed severe coryza and itching from the iodides. The KI was discontinued and a type of organic iodine substituted, namely, Organidin (Wampole Company, 400 Fairmount Avenue, Philadelphia, Pennsylvania) 10 drops t.i.d. This is an iodine organically compounded by reaction with glycerin purified to increase the absence of elementary iodine and its inorganic forms. Two other previous cases of iodine sensitivity in sporotrichosis have continued therapy with this product without further difficulty and successful cure of the disease. In addition, the ulcer received x-rays to the lesion, a total dosage of 2200 roentgen units, using 200 kilovolts, 20 milliamperes, 50 cm. target skin distance, 8 × 8 cm. field with a Thoreus filter. The generalized eruption disappeared within 2 weeks. The original ulcer was 85 % improved within 3 weeks and was completely healed at 5 weeks. No recurrence was noted during a 6-month follow-up.

Comment

We believe this case aptly illustrates the fact that sporotrichosis, like syphilis, hides under many masks.

One can only speculate as to the difficulty encountered in culturing our case. Attenuation of the fungus by penicillin is unlikely. Possibly local tissue enzymes released at the time of excision could have altered growth conditions. We wish to emphasize the importance of suitable animal inoculation when a deep fungus infection is suspected but cultures are not satisfactory.

We believe that the concomitant regression of the primary and secondary lesions corroborated the diagnosis of sporotrichosis with sporotrichid.

Summary

A case of sporotrichosis with sporotrichid is presented. Satisfactory cultures were obtained only after animal inoculation. Cure resulted from use of Organidin, an organic form of iodine, when a severe sensitivity occurred to KI. The fact that sporotrichosis has many clinical forms is emphasized.

Résumé

Un cas de sporotrichose, avec sporotrichides vient d'être présenté. Des cultures satisfaisantes ont été obtenues seulement après inoculation des animaux. La cure à l'Organidin, une forme organique de l'iodine, à été proposée quand survient une sérieuse sensibilité à KI.

On insist sur le fait que la sporotrichose à des nombreuses formes cliniques.

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Un Précurseur de Pasteur

J. B. F. Bulliard

1752—1793

par

E. J. Gilbert

(2.5.1952)

Cette notice sommaire est destinée à rappeler aux biologistes et aux milieux médicaux la vie de Bulliard et sa découverte de la véritable cause de la rouille des céréales, ses recherches sur la conservation des denrées alimentaires par l'ébullition, sa réfutation expérimentale des opinions ,,de ceux qui soutiennent que la putréfaction donne naissance à des êtres organisés'', et sa preuve expérimentale, d'une élégance toute pastorienne, qu'il n'y a pas ,,jusqu'au une moisissure qui ne soit le produit de la graine d'un individu de la même espèce''. Justice ne lui a jamais été rendue, et son biographe anonyme de 1839, pouvait déjà écrire: ,,On se sert souvent de ses travaux sans le citer''.

La vie de Bulliard est emplie de mystère. Les dictionnaires biographiques les plus anciens, comme les plus récents, sont, en bien des points erronés, en particulier en ce qui concerne sa date de naissance et celle de sa mort. Des recherches longues et difficiles m'ont permis de découvrir nombre de documents manuscrits, inédits, en particulier les actes d'état-civil, des documents notariés, etc, et beaucoup de textes anciens.

Bulliard Jean Baptiste François, à tort prénommé *Pierre*, naquit le 24 novembre 1752, à Aubepierre en Barrois (France). Il était le treizième et dernier enfant de François Bulliard et Elisabeth Tripier. Il avait moins d'un an lorsqu'il perdit son père, et à peine deux lorsque sa mère mourut. Ses tantes prirent soin de son éducation.

Après avoir achevé sa rhétorique au collège de Langres, il retourna dans sa famille, et dès lors se livra à la chasse avec passion ,,Habile oiseleur'', il avait formé une collection d'oiseaux qu'il empaillait lui-même. Et déjà, il s'était constitué un herbier considérable.

Des personnes qui avaient suivi ses études, lui firent obtenir un emploi à l'abbaye cistercienne de Clairvaux, vers 1771. Il y entreprit des études d'anatomie et de botanique, et y acquit une grande habileté dans l'art de peindre les plantes.

Dès 1775, on le trouve à Paris étudiant la médecine et la botanique, perfectionnant ses connaissances en dessin, et apprenant à graver sous François Martinet, habile peintre et graveur.

Il épousa, à une date difficile à préciser. Angélique Marie Gabrielle de Voulges. Celle-ci étant morte (1786?) sans lui laisser d'enfant, il se maria en seconde noce, le 3 février 1787, à Marie Adelaïde Lemercier, dont il eût deux filles: Anne Adelaïde Joséphine, née le 8 juillet 1788, et Marie Lucile, née le 11 janvier 1790. Il mourut de maladie le 29 septembre 1793 (= 8 vendémiaire de l'an Deux), dans son appartement de l'île Saint-Louis, à Paris, à l'âge de quarante-et-un ans.

Bulliard, qui, en peu d'années, a réalisé une oeuvre capitale pour la flore française, doit être considéré comme le principal fondateur de la Mycologie, son esprit scientifique étant proche du nôtre.

Parfait observateur, il a été le premier qui ait trouvé et expliqué la véritable cause ,,du charbon des blés'', ou rouille des céréales, ,,une plante de la famille des champignons'', et le mode de propagation de cette maladie. ,,Les graines de la réticulaire des blés, écrit-il, portées par les vents sur les épis encore dans leur fourreau attendent que les bales de ces épis se développent; elles s'insinuent entre les valves dont les organes de la fécondation sont entourés, pénètrent jusqu'aux grains encore tendres et mucilagineux, s'y enracinent, s'en approprient les sucs nutritifs, s'y développent, et donnent ensuite naissance à une prodigieuse quantité de graines d'un brun noirâtre, si fines qu'elles ressemblent à du charbon réduit en poudre, de là est venu qu'on a donné à cette réticulaire le nom de *charbon* . . . Voilà ce dont je me suis bien assuré par nombre d'observations faites et répétées avec le plus grand soin, tant au microscope simple, qu'au microscope composé. Quoique personne, à ce que je sache, n'ait eu connaissance jusqu'ici de la véritable cause du *charbon* . . .''.

Il s'était aussi longuement attaché aux problèmes de la fécondation des champignons, problèmes qui dépassaient les possibilités des naturalistes du temps, et n'ont guère reçu de solutions qu'en notre XXe siècle.

Etonnant expérimentateur, il avait réussit à cultiver une trentaine d'espèces de champignons, et, un siècle avant les découvertes cruciales de Pasteur, il avait résolu avec bonheur le problème de la génération spontanée, au moyen d'expériences d'une rigueur toute pastorienne, expériences que nous allons relater et que Pasteur, à ses débuts, ne put ignorer.

,,J'ai dit, écrit-il, que j'espérais prouver jusqu'à l'évidence qu'il n'y avait pas jusqu'à une *moisissure* qui ne fut le produit de la graine d'un individu de la même espèce; je vais en effet en mettre la preuve sous les yeux de mes lecteurs, en leur indiquant tous les moyens de répéter les expériences sur lesquelles cette preuve est étayée; ces expériences méritent la plus sérieuse attention, surtout de la part de ceux qui soutiennent que la *putrèfaction* donne naissance à des êtres organisés . . .

,,Quoique bien persuadé qu'un grand degré de chaleur devait faire perdre aux graines des mucors la faculté végétative, j'ai voulu m'en assurer par des faits et ne laisser aucune incertitude à ce sujet. J'ai fait tremper de la mie de pain dans de l'eau, j'en ai fait une espèce de bouillie, je l'ai saupoudrée d'une grande quantité de graines de mucors, j'y ai même mêlé diverses substances sur lesquelles il était venu des mucors de différentes espèces; j'ai fait entrer cette bouillie dans six fioles à médecine, j'ai arrangé ces fioles dans une grande chaudière sur un lit de foin; elles étaient plongées dans l'eau de manière à ce qu'il n'y eut que les goulots qui en fussent dehors; j'ai fait bouillir l'eau pendant une heure; j'ai ensuite étendu du suif sur des morceaux de papier dont j'ai recouvert deux de ces fioles pendant qu'elles étaient encore dans l'eau bouillante; je les en ai retirées, et en ai entortillé les goulots de plusieurs doubles de papier enduit d'une eau de gomme très épaisse; j'ai laissé les quatre autres fioles débouchées; dans deux de ces dernières j'ai fait entrer de la graine de mucors, et je n'en ai point mis dans les deux autres. Au bout de cinq jours les deux fioles dont j'avais laissé les goulots ouverts, et dans lesquels j'avais fait entrer de la graine de mucors se sont trouvées remplies de moisissure dont les pédicules étaient d'une longueur extraordinaire; les deux autres dont j'avais aussi laissé les goulots ouverts, mais dans lesquels je n'avais point fait entrer de graines, ont eu seulement quelques petites taches de moisissure; pour ce qui est de celles dont j'avais soigneusement couvert les goulots, au bout de deux mois il n'y avait pas plus d'apparence de moisissure que le premier jour; cette expérience vraiment curieuse je l'ai répétée plusieurs fois, je l'ai variée, j'en ai toujours obtenu les mêmes résultats, avec cette seule différence que les fioles dont je laissais les goulots ouverts, et dans lesquelles je ne mettais pas de graines, n'avaient quelquefois qu'une ou deux petites taches de moisissure, et quelquefois n'en avaient pas du tout; sans doute parce que le goulot de ces fioles, étant étroit et allongé, la petite quantité de graines que l'air extérieur y apporte, au lieu de pénétrer jusqu'au mélange contenu dans ces fioles, se colle aux parois du verre. . . . . Indépendamment du jour que doivent jeter sur la cryptogamie les expériences dont je viens de rendre compte, elles pourront encore être d'une utilité plus générale: en enseignant à s'opposer par des moyens certains, à ce que sur une infinité de substances il vienne de la moisissure".

La mort, survenue avant l'heure, n'a pas permis à ce savant d'une activité prodigieuse de publier les autres études expérimentales qu'il avait entreprises.

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Ainsworth G. C. and Sampson K. — *The British Smut Fungi* (*Ustilaginales*). Vol. pagg. 137, 30 figg. The Commonwealth Mycological Institute, Kew, Surrey. 1950. Bound.

About sixty years after the Plowright's Monograph on British Rusts and Smuts, a new monograph on British Ustilaginales is now published by the senior author, formerly of the staff of the Commonwealth Mycological Institute of Kew, and the junior author, well known student of the biology of smut fungi.

The British flora of Ustilaginales is quite reduced in absolute number of species (64 at all), but most of the widely diffused, continental species, are represented in the islands.

The classical concepts of morphological foundation of the smut species are taken as major premises for the revision, the Authors being conservative in the taxonomy as well as on nomenclature, with the exception on the specifical identity of *Ustilago tritici* with *U. nuda* (under the former binomial) and of *U. kolleri* with *U. hordei* (under the latter binomial); only three new combinations are proposed in the text. One of the outstanding feature of this monograph, then, is the equilibrium between the opposite tendencies to split up the old species on the base of biological characteristics, and to retain the older, morphologically founded species.

An unusual feature of this monograph is the addition of notices not only on spore germination, but also on infection of the host plants and racial specialization, with an exhaustive bibliography. A number of subjects of comparatively recent development is sumarized in the introduction to the taxonomical treatment, stimulating the interest of biological students on smut fungi.

This book fully maintain the high standard of quality, in content as in presentation, which has allways characterized the english work in mycology.

R. Ciferri

South-American blastomycosis-A review.

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The study of South-American blastomycosis, not very properly called Brazilian blastomycosis, is growing more and more important on account of the increasing occurrence of this mycosis, and, most of all, because of the seriousness of some its anatomo-clinical forms. This infection, of fungous nature, has been studied very well by Brazilian authors, being nevertheless little known by American and European dermatologists and tropicalists. This is the reason why we propose to discuss the subject, presenting a general survey upon the actual standing of the matter, with special reference to the Brazilian contribution to the study of the above said mycosis.

South-American blastomycosis, also called Brazilian blastomycosis, paracoccidioidomycosis, paracoccidioidal granulomatosis, Lutz's disease, Lutz-Splendore-Almeida's disease, Almeida's disease, malignant ganglionar granuloma of blastomycetic origin, has been observed for the first time by Lutz (77) in Brazil, who called attention to the lesions sited at the patient's mouth. The study of the parasite's morphology in the lesions caused Lutz to foresee some differences from the *Coccidioides immitis*, agent of Posadas-Wernicke's disease.

There follow the works of Splendore (122—127), all of them well documented, which contribute to a better knowledge of the microbiology of South-American blastomycosis agent. In 1912 he suggested the name of *Zymonema brasiliensis* for the microorganism causing that form of deepset mycosis, that is by now widespread in Brazil. In 1910, bearing reference to the cases of blastomycosis observed until then, Splendore (123) stated, in a report presented to the 4th. Latin-American Medical Congress: "these cases, all of them more or less alike, are characterized by the lesion being sited primarily at the mucous membrane of the mouth." There follows, at page 8 of his work: "in two cases the onset has been precisely at the angle of the mouth, and in the third one, at the gums. The patients complained frequently of difficult swallowing; some had an enormous salivary secretion; almost all of them presented an extreme weakiness that increased steadily. Some died following to extreme caquexia; others are still alive, but they don't show any noticeable improvement. None of the remedies applied locally has given any result up to now."

Splendore (123) says, referring to Lutz's work: "as to my cases, of which there were three, I have one whose examination was limited to the material to that isolated by Dr. Lutz; the colonies presented the characteristic filamentous aspect, especially on glycerinated agar, that has been compared by some authors to the hide of white mice."

A fundamental work of Splendore (126) has been published in 1912 in Italy, bound in a volume commemorating the 25th anniversary of Prof. Angelo Celli's teaching career. In this work Splendore studies 4 cases of paracoccidioidal granulomatosis, describing with accuracy the clinical characteristics of the fungous infection. At page 422 he says, textually: "trattasi di una malattia micotica speciale dominante in Brasile, la qual puó essere classificata fra le cosi dette blastomicosi generalizzate descritte principalmente nell'America del Nort, ma, per la localizzazione specifica delle sue lesione nella cavitá della bocca, non trova fino ad ora, riscontro nella patologia di altri paesi."

The clinical description made by Splendore of paracoccidioidal granulomatosis is perfect, and those who study carefully the disease are apt to verify that the Italian bacteriologist is precise in all his works where he analyzes the clinical picture of the cases he observed.

There is no doubt that we are indebted to LUTZ and SPLENDORE for the study of the first cases of blastomycosis observed in Brazil. CARINI (39), LINDENBERG (74), RABELO (108), PEREIRA and GASPAR VIANA (104), MONTENEGRO (86), CASTRO CARVALHO (41), DIAS DA SILVA (119—120), GOMES DA CRUZ (57), PORTUGAL (107) and KEHL (60), ranging from 1908 to 1915, reported other cases of South-American blastomycosis; nevertheless, it was SPELDORE's work that imparted the big impulse to the clinical and mycological study of this fungous infection.

It wouldn't be logical to filiate the cases of SPLENDORE's to other mycotic diseases, as for instance, GILCHRIST's disease, which occurs only exceptionally in Brazil. One of the most common characteristics observed in patients of South-American blastomycosis is the site of the lesions at the mucous membrane of the mouth and pharynx, this being not the case in GILCHRIST's disease.

Since 1930 most of the mycologists make use of the following designation to name the agent of South-American blastomycosis: *Paracoccidioides brasiliensis* (SPLENDORE, 1912) ALMEIDA 1930. With this denomination ALMEIDA (3) revalidated and described again the species *brasiliensis* of SPLENDORE, ascribing the aforesaid microorganism its proper place in a new genus, *Paracoccidioides*. In 1936 CIFERRI and REDAELLI (42) put the genus *Paracoccidioides* in a new family, *Paracoccidioidaceae*, removing it still further from the genus *Coccidioides*. FONSECA FILHO (52), by accepting the differences between *Coccidioides immitis and Paracoccidioides brasiliensis*, tries to invalidate the species created in 1912 by SPLENDORE, in order to give the agent of South-American blastomycosis the new generic denomination *Lutziomyces*, with the species "*histoporocellularis*". SIMOES BARBOSA (25) proved this denomination to be superfluous and illegitimate, and therefore it should be discarded. HABERFELD (59) described the species "*histoporocellularis*" not knowing about SPLENDORE's work published in 1912; he knew only about this author's first publication (1909), and thus he says at page 81 of his monograph: "we may state that the description of the microscopic aspect of the cultures of the germ that is at present catching our attention has been made by LUTZ and SPLENDORE, the first to observe the disease in Brazil, and it so comes that few facts remain worthy of any special interest." The species "*brasiliensis*" created by SPLENDORE is absolutely valid and is not contrary to any disposition of the international rules of botanic nomenclature.

CONANT and HOWELL, Jr (43—44) suggest the generic denomination *Blastomyces* for the agent of South-American blastomycosis, including there both species, "*dermatitidis* and "*brasiliensis*". Even if both species are proper or akin in their morphobiological characters, the generic denomination *Blastomyces* should be discarded, because it has been suggested by CONSTANTIN and ROLAND for a saprophyte fungus that has no likeness whatever to the agent of South-American blastomycosis. Some authors prefer to substitute it, the genus *Gilchristia*, posterior to *Paracoccidioides*. As ALMEIDA (19) has already shown, the most logical would be to accept the genus *Paracoccidioides*, with the species "*dermatitidis*" and "*brasiliensis*". Up till now we still call the agent of South-American blastomycosis by the name of *Paracoccidioides brasiliensis* (Splendore, 1912) ALMEIDA 1930.

The species "*cerebriformis*.' and "*tenuis*", created by MOORE (89—90) seems to be synonymous to *Paracoccidioides brasiliensis*. This fungus, like many others, undergoes many variations so that single morphologic characters, macroscopic and microscopic, do not wholly fulfill the purpose of accurate separation of species. What we must do is to amplify the original description of *Paracoccidioides brasiliensis*, founded on more data, by studying more samples and noticing their morphologic variations as well as the biochemical and pathogenicity ones, in order to become better acquainted with the microorganism.

There are two other fungi agents of special clinical forms of blastomycosis observed in Brazil that are in need of more accurate studies. We mean *Glenosporella Loboi* and *Glenosporopsis amazonica*. In 1933 JORGE LOBO (76), professor of Dermatology at the Faculdade de Medicina de Recife (Pernambuco, Brazil), described a form of blastomycosis most interesting because of its anacomo-clinical aspect (cheloid lesions); he underwent many difficulties about isolating a fungus from cutaneous lesions; this fungus was later on found by FONSECA FILHO and ARÊA LEÃO (53), to pertain to the genus *Glenosporella*, species "*Loboi*". The aspect of the lesions is completely different from that observed commonly in the majority of cases of South-American blastomycosis. It should be noted that the patient Lobo studied came from Amazonas. The aspect of the parasite in the lesions is characteristic. The great numbers in which they are present in histologic preparations are apt to draw the pathologist's notice. AMADEU FIALHO (47—48) studied minutely the histopathology of "Jorge Lobo's blastomycosis"; at quite a near past ARÊA LEÃO and others (23) found that *Glenosporella Loboi*, when grown on blood-agar at 37° C, shows round, globose cells of various sizes, that reproduce themselves by simple or multiple or multiple budding. These morphological types observed by ARÊA LEÃO and others (23) show that there are resemblances between *Paracoccidioides brasiliensis* ans *Glenosporella Loboi*. We think that the cheloid lesions first observed by Lobo should have an individual clinical standing, but we do believe *Glenosporella Loboi* to be identical with *Paracoccidioides brasiliensis*. At the utmost, it might be considered as another species within the genus *Paracoccidioides*. The natural habitat of this microorganism seems to be the vegetal kingdom. According to the environment in which it grows its morphobiology may be completely altered. The very ways by which the organism can be infected can determine pictures very polimorphous, this being observed in a great many infectious agents.

Another fungus, agent of a special form of blastomycosis, has been described by FONSECA FILHO (54) under the denomination of *Glenosporopsis amazonica* (n.g., n.sp.). Up till now there is only one case of such a type of blastomycosis reported in medical literature. In spite of being macroscopically different from *Paracoccidioides brasiliensis*, we have our doubts as to the validity of the genus and species denomination. At present we are studying cultures of *Glenosporopsis amazonica* in order to make our mind definiterly up on the subject. It is our wish to underline the following: *Paracoccidioides brasiliensis*, under varying circumstances, undergoes deep changes in its morphobiological characters, this causing polimorphous lesions that present histological structure also varying much. This little introduction, necessary especially to those that wish to know more about the aforesaid problem, precedes the general study of the habitat and morphobiology of *Paracoccidioides brasiliensis*.

Cordeiro de Azevedo (24) in his thesis also concludes that *Paracoccidioides brasiliensis* is identical to *Glenosporella Loboi* and *Glenosporopsis amazonica*.

Everything points to the fact that *Paracoccidioides brasiliensis* should have its natural habitat in the vegetal kingdom. No positive culture has as yet been obtained from soil, graminacea, or vegetable seeds, but we succeeded in cultivating the *Paracoccidioides brasiliensis* in parasitary forms, and also from culture kept in agar-Sabouraud: we inoculated the abovesaid material in a great variety of vegetable grounds, as for instance "peroba" [*Aspidoperma polyneuron*, "ipê" (*Tecoma chrysostricha*, Mart.)], coffee leaves, pieces of "jacaranda" (*Machoerium villosum*), etc. (11). In this manner we prove, though not directly, that the reservoir of *Paracoccidioides brasiliensis* is the vegetal kingdom. Thus the plants act as intermediary hosts for human infections. *Paracoccidioides brasiliensis* lives as a saprophyte on the soil and vegetals, and is carried into the human organism via little wounds caused by a vulnerating body or agent of vegetal procedence; in the great majority of cases *Paracoccidioides brasiliensis* is carried into the organism via the mucous membrane of mouth and pharynx: the patients refer to the habit, we should say vice, of cleaning their teeth with pieces of plants, or of chewing leaves and tree rinds of many kinds. As it is, this vice is extra-ordinarily widespread among the rural populations of the states of Brazil.

Some other times the parasite makes its way through the anus in patients that usually make their anal toilet by the means of leaves. Up till now the presence of *Paracoccidioides brasiliensis*, in inferior animals couldn't be proved, so that one can partially remove that possibility of these animals functioning like reservoirs or bridges to the infection. It seems to us that a great number of patients acquire the infection through inhalation of infecting elements. The respiratory tracts represents, in blastomycosis, one of the most common ways by which the infecting agents enter the body, according to the view-points of Mattos Barreto and Lacaz.

At its parasitary phase *Paracoccidioides brasiliensis* presents a characteristic aspect. It is always found abundantly in the material from lesions, presenting itself under the form of round cells, with a very refringent double membrane. Slides of tissues, stained by Perdrau's method, show two outstanding forms of the fungi's reproduction, well studied by Bogliolo (30—33) and AMadeu Fialho (49). One of these is by simple budding, being the other and more frequent one, cryptosporulation, as called by Ciferri and Redaelli (42). At a certain phase of its evolution cycle, *Paracoccidioides brasiliensis* presents its chromatin finely chopped into little chromidia that function as "potential spores". Afterwards, these chromidia site themselves at the cell's periphery; they press the cell's membrane dragging some cytoplasm along. At this phase we can see the *Paracoccidioides brasiliensis* with a great many new cells; these buds are formed by a mechanism different from that of the clan or true budding.

Cultures of *Paracoccidioides brasiliensis* can be grown at room temperature. Inoculating *Paracoccidioides brasiliensis* at 37° C. in blood-agar or chocolate-agar, after 8 to 12 days little colonies are seen to have developped; they are easily removed from the medium, and its microscopic morphology is identical to that observed in the tissues. At room temperature, after 20 to 40 days, the *Paracoccidioides brasiliensis* presents a wooly aspect, filamentous; nevertheless, there are big variation according to the medium where the fungus is grown.

Microscopic examination of these colonies shows septate filaments, with more or less chlamydospores. One may easily change a filamentous colony into a culture of yeast-like type. This is obtained by inoculating the material on blood-agar or chocolate-agar at 37° C. This change is reversible, as ALMEIDA, LACAZ and FERNANDES (16) have demonstrated.

Paracoccidioides brasiliensis grows relatively well at room temperature on various nutrient media; LACAZ, AZEVEDO and BOLOGNANI (61) have suggested or its isolation the media of blood-Sabouraud, chocolate-Sabouraud and spleen-extract-Sabouraud. From the cultural viewpoint, *Paracoccidioides brasiliensis* differs from *Blastomyces dermatitides* especially because this last fungus grows much more luxuriously than the first one. On certain media *Blastomyces dermatitidis* grows upon the tube's walls.

The biochemical activities of *Paracoccioides brasiliensis* are practically non-existent, and they don't present any big interest for its identification. From the experimental viewpoint the only animal that shows some receptivity to the fungus is the guinea-pig, when inoculated by testicular route. Even so, the injection does not spread to other organs, as has been demonstrated by the works of MONTENEGRO (88), ALMEIDA (7), FIALHO and PADILHA GONÇALVES (50), and PERYASSÚ (105).

An experimental work of PERYASSÚ's has demonstrated that animals with SRE blocking are more sensible to infection; in these cases the histological picture differs from that observed in normal animals. In cases of SRE exaltation, there overrules a picture of tissular hyperergy, whereas in cases of blocking it is anergy that dominates.

For a long stretch of time *Paracoccidioides brasiliensis* has been mixed with *Coccidioides immitis*; SOUZA CAMPOS and ALMEIDA (35), especially this latter Brasilian author, in a series of works (2—20) have shown the differences between the fungi. The great majority of mycologists accept those differences today.

Statistical data

According to HABERFELD (loc. cit.) we may talk of a true blastomycetic endemy in the state of São Paulo (Brazil). PIRAGIBE NOGUEIRA DA SILVA (95) in his doctorate thesis, reports that South-American blastomycosis is a subject of general clinics, being so important in Brazil as to be put together with the country's tuberculosis and syphilis also. Some cases of blastomycosis in São Paulo are reported in ARANTE's thesis (22).

The cases of blastomycosis have increased among us, unhappily enough, the infection that was practically rural, has attained suburban and sometimes urban areas, striking individuals pertaining to any calling in life. South-American blastomycosis has been reported with greater frequency in Brazil, especially in the state of São Paulo, where it has been most thoroughly studied.

In Argentine, LLAMBIAS, TOBIAS and NIÑO (75), MAZZA and others (82), NEGRONI and others (92), SEMINARIO and others (117), MAZZA and PALAMEDI (83), NIÑO and others (93), CANAL FEIJOÓ others (36), PIERINI (106), MOSTO (91), MARENGO and others (81), reported cases of South-American blastomycosis. Some of them are reported as blastomycosis by special yeasts, but an accurate study of the same leads us to believe them to be paracoccidioidomycosis.

There were also other cases of blastomycosis observed in Uruguay and Paraguay.

Up to August 31, 1945, there were registered 750 cases of blastomycosis at the Department of Mycology of the Faculdade de Medicina da Universidade de São Paulo. Those cases were studied by Almeida, Lacaz, and Cardoso da Cunha (15); the highest percentage of cases of the infection occurs in white people; second come yellow people. Cases of paracoccidioidal granulomatosis are very frequent among Japanese, especially farm people. The disease strikes negroes and mulattoes alike. Rural workmen pay a heavier tribute to the infection. Nevertheless, we are always seeing cases in individuals that never left the big cities. This shows that there is a spreading of the infection from rural areas to urban and suburban centers. It has been happening slowly, so that it becomes more and more necessary that a study be made which covers all these aspects of the problem.

In 1948—49 Almeida and col. (20) registered 964 cases of blastomycosis in the Department of Mycology of the "Faculdade de Medicina de São Paulo"

Paracoccidioidosis in Brazil is a true endemy that strikes especially adult rural workers.

Anatomo-clinical forms of South-American blastomycosis

The following chart will serve us as a guide in the anatomoclinical study of this mycosis, in an attempt of classification.

- *Anatomo-clinical forms of South American blastomycosis*
  - 1. *Tegumentary forms* (Cutaneous-mucous)
    - Papulous dermo-epidermitis
    - Papulopustulous dermo-epidermitis
    - Tuberous dermo-epidermitis
    - Vegetative dermo-epidermitis
    - Escrophulodermatitis
    - Diffuse hypertrophic granuloma of lips
    - Muriform ulcerous stomatitis
    - Concealed paracoccidioidic amygdalitis
  - 2. Ganglionary forms
  - 3. *Visceral forms, and other organs*
    - Spleen. Liver. Intestines. Pancreas
    - Lungs
    - Bones
    - Glands of internal secretion
    - Genitalia
    - Nervous system
    - Kidneys
  - 4. *Mixed forms*
    - Lymphatic-tegumentary
    - Lymphatic-visceral

By this scheme one can see how varying the clinical aspects of paracoccidioidal granulomatosis are; there may also occur localized forms on the mucous membrane of mouth, or else very grave widespread septicemic processes.

1) *Tegumentary lesions* (Cutaneous-mucous) — these ones have been well studied by Aguiar Pupo and Cunha Motta (1), Oliveira Ribeiro (113), Rafael da Nova (96,), Tella (128), Manzoli (80) and others. The primary cutaneous form of blastomycosis is rare; dermatitis lesions are almost always secondary Ramos e. Silva (111) state that blastomycosis in primary cutaneous processes may resemble dermic sarcoid, lupoid sycosis, and dermatitis verrucosa. Terra and Barros Barreto (129) reported a case with primary site at the mucous membrane of the nose, which afterwards ulcerated, present-

ing a frankly vegetating background. We had the chance to observe a case of blastomycosis with upstart at the lid, followed by a sattelite adenitis. A lesion commonly observed in blastomycosis is muriform ulcerous stomatitis (AGUIAR PUPO), also called "papulo-erosive form" by RAFAEL DA NOVA (loc. cit.) It is a stomatitis of slow evolution, exulcerated, whose background shows minute red granules, like those of a mulberry. RAFAEL DA NOVA (loc. cit.) has described the so-called "occult paracoccidioidal amygdalitis", where the macroscopic aspect does not draw suspicion towards mycotic lesions; nevertheless it is accompanied by tributary ganglionar lesions, because histopathologic examinations have shown the presence of paracoccidioidal granulomatosis. The macroscopic aspect is that of a chronic amygdalitis with sclerosis and increase of tissue's consistence; there are marked peri-tonsillary adherences.

2) *Ganglionar lesions* — it is difficult and practically almost impossible to separate tegumentary from ganglionar forms, owing to the fact that the latter follow the former. The ganglions from the neck are almost always the first to be struck; this is easily explained on account of primary blastomycetic lesions being generally at the mucous membrane of the mouth and pharynx. At the beginning the ganglions are firm and painful; as time passes they soften, adhere to the skin and ulcerate, discharging a caseous pus, full of parasites. The ganglionar tumefaction especially at the neck is, accordingly, one of the alarm signs of the infection. Dental apical granulomae, of paracoccidioidal origin, may give rise to a specific adenopathy, of cervical site, as has been shown by BOGLIOLO (31). There are cases of intra-abdominal adenopathies reported, that lead to errors in diagnosis: they resemble peritoneal tuberculosis or a malignant tumor of the abdominal cavity. Some of the widespread forms may be mistaken for HODGKIN'S disease. CARINI (40) studied a case of abdominal blastomycosis that started with vomitings, fever and pains at the right illiac fossa. An appendicitis was diagnosed clinically. On operation a tumor was noted between the mesocolon leaves, invading the retro-peritoneal lymphatic ganglions, reaching up to the renal region. There were no lesions to be observed in the appendix. The biopsy of one of the ganglions revealed a paracoccidioidal granulomatosis. Tuberculous lesions may be associated with ganglionar blastomycetic processes, according to reports made by PADILHA GONÇALVES (98)

3) *Visceral forms, and other organs* — primary visceral forms of blastomycosis are rare among us. As a rule they represent a generalization of the mycotic process. There may be lesions of the intestines, liver, spleen, and all of the lymphatic system; in such cases *Paracoccidioides brasiliensis* may be isolated from blood; positive hemocultures were already obtained by MIGUEL PEREIRA and GASPAR VIANA (104), MONTENEGRO (87), ROSENFELD (114), ALCANTARA MADEIRA, LACAZ and FORATTINI (78). The spleen is by far the organ most apt to be struck. CUNHA MOTTA (45—46) states that in 98 % of cases with generalization that organ was struck with multiform lesions, microscopically and macroscopically. Owing to its richness in lymphoadenoid tissue the intestines present a marked predisposition to blastomycetic infection, according to HABERFELD (loc. cit.). GASPAR VIANA (132) described with great accuracy the appendiculary lesions of paracoccidioidal granulomatosis. Pancreatic localization has also been described (55). Pulmonary lesions are relatively frequent in South-American blastomycosis, and have been the subject of interesting studies by BASGAL (26), CUNHA MOTTA (46), PADILHA GONÇALVES and BARDY (97), MOACIR SANTOS SILVA (115—116), ALMEIDA and LACAZ (9), and FIALHO (loc. cit.). Such lesions may be associated with tuberculosis. In Argentine, FLAVIO L. NINO (94) found a high incidence of pulmonary lesions in patients of blastomycosis; this fact has been observed also by FIALHO in Rio de

Janeiro. Thus, in 22 necropsies performed by Amadeu Fialho (loc. cit.), and in three others that were handed over to him by colleagues of his, there were blastomycetic lesions in the lungs; 12 % of the cases had associations with tuberculosis. Versiani (130), in Minas Gerais, met often with pulmonary localization.

In cases of pulmonary localization the radiologic picture of the lungs is very much like that found in tuberculosis; the lesions may spread or be limited to the bases. A correct diagnosis can be obtained only by examination of sputum. Among the glands of internal secretion, the adrenals are struck the oftener in cases of generalized blastomycosis; thus it was reported by Pena de Azevedo whilst describing a case (102). Flaviano Silva (118) reported a case where the thyroid was very much involved. There have also been reported cases of osteo-articular lesions. Nogueira da Silva (loc. cit.), in his doctorate thesis, reports a case of osseous lesions at the illiac bone, besides other manifestations. Camargo and Gonzaga (34), Lacaz (63), Laport and Fialho (72), Versiani (130), Monteiro de Barros (85) and Rafael de Lima Filho (73) have reported cases of South-American blastomycosis with osseous manifestations. The nervous system is seldom involved. Penna de Azevedo (101), Maffei (79), Gonzales and Boggino (58), Lacaz, Assis and Bittencourt (66) have reported cases of paracoccidioidal granulomatosis with meningoencephalic manifestations. The case reported by Lacaz, Assis and Bittencourt consisted of blastomycetic lesions of mouth and lungs, with medular repercussion, that gave rise to a meningo-radiculo-medullar syndrome; it almost desappeared with sulphonamidic therapy.

Ways by which the organism enters the human body

In almost all cases, *Paracoccidioides brasiliensis* enters the human body by way of the mucous membrane of mouth, pharynx and respiratory tredet causing processes of stomatitis or else amygdalitis or pneumonitis. Some times there are lesions not at the mouth and pharynx, but at the larynx only; in such cases paracoccidioidal laryngitis is almost always secondary to pulmonary manifestations of blastomycetic origin. The initial lesions of paracoccidioidosis may also be localized at the skin, ano-retal mucous membrane, mucous membrane of the nose, and even the conjunctival mucous membrane. Primary cutaneous lesions are rare indeed. Sodré and Cerrutti (121), Monteiro and Amadeu Fialho (84) reported two cases of primary ano-retal blastomycosis. In 1930 Belfort (27) reported a case of conjunctival blastomycosis. Pulmonary lesions of South-American blastomycosis may develop primarily at the lungs after inhalation of *Paracoccidioides brasiliensis*. Nevertheless, in almost all cases the lesions are the result of a lymphatic or hematogenic dissemination of a focus sited elsewhere.

Diagnosis of south-American blastomycosis

The following chart, taken from a work of Lacaz's (64), gives us an idea of examinations and tests that should be performed in a case of South-American blastomycosis.

1) Mycological diagnosis
    a) Microscopic examination
    b) Isolation
    c) Identification
    d) Inoculation
2) Anatomo-pathologic diagnosis

3) Immuno-allergic diagnosis
  a) Fixation of the complement test
  b) Serologic tests
  c) Intra-dermo reaction with paracoccidioidin

4) Auxiliary tests
  a) Radiologic examination
  b) Hemogram and hemosedimentation
  c) Myelogram
  d) Tests of hepatic function
  e) Examination of liquor (when meningo-encephalic lesions are suspected

1) *Mycological diagnosis* — Demonstration of *Paracoccidioides brasiliensis* in fresh preparations, stained or not, presents no difficulties, because the microorganism has its characteristic morphology whether in pus, sputum, or any secretion whatever. Catenulated forms may be found, facilitating diagnosis very much. The ganglions are very rich in parasites. With material taken from lesions either of mouth or pharynx, smears are made that are stained by Giemsa's method; parasites are thus easily recognized; this procedure has the advantage of keeping material always at hand for examination whenever one should be pleased to do so.

The isolation of *Paracoccidioides brasiliensis* can be made within a period of seven to ten days, in blood-agar or chocolate-agar at 37° C, as ALMEIDA and FERNANDES (10) have shown. When inoculated on the classic Sabouraud-glucose medium, at room temperature, the fungus develops slowly, starting from twenty to thirty days after inoculation. In cases of generalized blastomycosis *Paracoccidioides brasiliensis* may be isolated from the blood. GASPAR VIANA (loc. cit.), MONTENEGRO (loc. cit.), ROSENFELD (loc. cit.), ALCANTARA MADEIRA, LACAZ and FORATTINI (loc. cit) have obtained cultures of *Paracoccidioides brasiliensis* from the circulating blood.

Inoculations of *Paracoccidioides brasiliensis* (pus, cultures, scrapes from mouth and pharynx, sputum) are to be made into guinea-pigs by testicular route.

Identification of said microorganism is done according most to its micromorphology, in tissues and culture.

2) *Anatomo-pathological diagnosis* — according to this side of the question South-American blastomycosis has been very well studied by CUNHA MOTTA (loc. cit.), AMADEU FIALHO (loc. cit.) and BOGLIOLO (loc. cit.). Other authors have contributed, like HABERFELD (loc. cit.), GASPAR VIANA (loc. cit.) and RAFAEL DA NOVA (loc. cit.), with most accurate data to a better knowledge of paracoccidioidal granulomatosis.

3) *Immuno-allergic diagnosis* — among the serologic tests performed for diagnosing South-American blastomycosis the one that presents any practical interest whatever is that of fixation of complement. We have already had some experience with this test, employing as antigen a filtrate in Sabouraud-broth of many samples of *Paracoccidioides brasiliensis*. The technique that proved best was that of KOLMER, slightly changed. The tests were made in the spinal fluid of patients with blastomycosis and the results can be verified in a thesis we made and published (62) in 1945. When correctly understood, together with clinical data, it shows its worth. Thus, patients with evolutionary lesions react intensely positive; when the case grows better through sulphonamidic treatment the reaction steadily decreases in positiveness, up to becoming negative. The fact that fixation of complement test may turn out negative does not imply that there is no chance of existence of a blastomycetic infection; we say so founded

on the fact that we have isolated *Paracoccidioides brasiliensis* from minute lesions in patients with negative reaction after treatment with sulphonamidic derivatives. The fixation of complement test must always be performed before any treatment whatever is begun, and whilst treating; its results should be controlled by other examinations (histopathologic examination and hemosedimentation); bearing in view the practical side of the question, we find that the presence of *Paracoccidioides brasiliensis* establishes without the shade of a doubt a diagnosis of paracoccidioidal granulomatosis.

Intradermic reactions have also been employed on diagnosing South-American blastomycosis. It was first employed by OLIMPIO DA FONSECA FILHO and ARÊA LEÃO (51). Afterwards, together with ALMEIDA, we have studied (11) this test employing as antigen a filtrate in Sabouraud-broth of many samples of *Paracoccidioides brasiliensis* grown during a few months at 24° C. — 28° C. Elated by the results obtained, together with ALMEIDA and CARDOSO DA CUNHA (11) we prepared 3 types of antigens, which were called paracoccidioidin I, II and III.

Paracoccidioidin I is a filtrate of 19 samples of *Paracoccidioides brasiliensis* in Sabouraud-broth, kept at 24° C. — 28° C. during 3 months. Paracoccidioidin II is obtained from 19 samples of *Paracoccidioides brasiliensis* grown on chocolate-agar. It consists of a suspension, made after shaking the samples with glass pearls, in a 0,5 % phenicated saline physiological solution. The suspension is easier to be obtained on account of the cerebral form of the colonies on the abovesaid medium, after heating to 80° C. during half an hour three consecutive days; it was then diluted in 1/10 and 5 % saline physiological solution. Paracoccidioidin III is a suspension of pus rich in *Paracoccidioides brasiliensis* (1:10 suspension in saline physiological solution) heated to 70° C. during half an hour three consecutive days.

From all these antigens the one that proved best for reaction was paracoccidioidin I. Results of the tests were read 24 to 48 hours after intradermic injection. We were able to see that, as a rule, in grave cases of blastomycosis with generalized and evolutionary lesions, the intradermic reaction was negative, thus proving the condition of anergy of the patients. Patients presenting general well-being reacted positively to the antigen: there was a slightly papuloes erythema. As one can readily see, the hypersensibility to the blastomycetic antigen is related to the body's resistance to infection. Patients with low or no sensibility to the antigen almost always present little resistance to the infection. It is our opinion that, as a single element on diagnosing, the reaction is worth but little, owing to the readiness with which one can find the parasites in the lesions.

Paracoccidioidin is now prepared by us employing Smith's medium: five samples of *Paracoccidioides brasiliensis* are grown during two months at room temperature, and then filtered.

VERSIANI (131) reports that intradermic reactions with coccidioidin performed in patients with South-American blastomycosis are always negative. The same goes for us and will be reported in a work still to be published.

N. F. CONANT has kindly sent us blastomycetin, prepared from *Blastomyces dermatitidis*. With this antigen we were able to obtain some positive results, thus being shown the group relations of the North-American and South-American blastomycosis (68).

4) *Additional tests* — among the additional tests that prove useful in cases of South-American blastomycosis we must cite: hemosedimentation (of high worth in controlling and curing the infection); the hemogram and myelogram; the radiologic and the spinal fluid examinations, the latter in cases

with meningo-encephalic lesions. Hemosedimentation, as we were able to verify together with ALMEIDA and CARDOSO DA CUNHA (13) is always increased in patients with blastomycosis that are still under treatment. While the lesions grow asunder, the rate of red corpuscles sedimentation falls. It seems to us that the increase in the rate of hemosedimentation is due to hyperglobulinemia, commonly to be observed in patients with blastomycosis. As concerns hemogram and myelogram in blastomycosis, we must cite the work of RAMOS, ORIA and PIO (110) published in 1939. Afterwares ADERBAL CARDOSO DA CUNHA (38) and the following results were obtained:

a) the mercuric bichloride that enters in the composition of Hayem's liquid determines the agglutination of red corpuscles in patients with blastomycosis;
b) however, this agglutination is not specific; it is perfectly possible that it occurs owing to the quantitative and qualitative changes of plasmic globulins;
c) in 16 cases of South-American blastomycosis out of 33 that were studied, there was a normochromic olygocytemia;
d) as a rule there is a great rate of leucocytes in grave cases; there is neutrophilia with a shift to the left, together with cytoplasmic and nuclear degenerative signs;
e) eosinophilia is frequent, with no dependence of verminosis;
f) atypic lymphocytes are frequent, same as histioid cells;
g) monocytosis frequently observed.

At the bone marrow there is following to be noticed: percentual decrease of every component of the eosinophilic sector; high values for lymphocytes, monocytes and plasmocytes; sometimes high increase of the sre elements.

An examination that is always to be made besides those is the radiologic examination, especially of the lungs, because it is apt to show the presence of lesions that go unnoticed at clinical examination. It seems to us that there are no specific radiological data that enable one to diagnose blastomycetic lesions of the lungs or bones. As a rule these resemble tuberculosis, but they take preferably to the bases and parahilar zones.

Treatment of South-American blastomycosis and the control of its cure

Thanks to OLIVEIRA RIBEIRO (112) the prognosis of South-American blastomycosis has vastly improved. The sulphonamidic preparations, especially sulphadiazine, have a noticeable fungistatic effect upon *Paracoccidioides brasiliensis*; it improves patients general condition much, leading them to clinical cure, whereas the true cure is a task of the organism's. Iodine preparations are not to be employed in the treatment of South-American blastomycosis.

Before sulphonamide preparations were used, patients of blastomycosis died inevitably, after a verying stretch of time, according to their organic resistance and the infection's anatomo-clinical form. With the employ of sulphonamide preparations, the lesions back at once, but the cure is only clinical: this is why relapses may occur in medical practise on laying of the drug.

Experimental works of PERYASSÚ'S (loc. cit.) have show that the sulphonamides act fungistatically upon *Paracoccidioides brasiliensis*. The same has been reported by us together with ALMEIDA and FORATTINI (12), and according to the studies of PELLEGRINO (100) and CARDOSO DA CUNHA (37). This is the reason why ALMEIDA (6) suggests that a stock vaccine should be employed together with sulphonamide preparations, thus stimulating the specific immunitary defenses.

According to PADILHA GONÇALVES (99) there is a 5 mg blood concentration of sulphadiazine to be maintained, in cases of blastomycosis, until the patient is clinically cured.

"In vitro", para-amino-benzoiç acid diluted till 1/1000 has a fungistatic action upon *Paracoccidioides brasiliensis*, after BIOCCA and LACAZ (28). Penicilin and streptomycin has no effect upon South-American blastomycosis, as was show by the experimental studies of ALMEIDA and others (14—67).

Tirotricin, after LACAZ, SANTOS SILVA and FERNANDES (65) has an inhibitory effect upon the fungu's growth, if it should be at its lag-phase.

Merthiolate has also a marked fungistatic action upon *Paracoccidioides brasiliensis*, according to ALMEIDA, LACAZ and FERNANDES (18).

At the present state of our knowledge of South-American blastomycosis' therapeutics, we recommend the following general rules of treatment:

a) general care (healthy food, vitaminotherapy, etc.);
b) removal of dental foci in cases of gun lesions, or of the mucous membrane of mouth;
c) treatment by sulphadiazine or sulphamerazine (1 g. every 5—6 hours) till clinical cure is obtained. Together with sulphonamide preparations by oral route, we may employ them by pulverization of lesions of mouth, pharynx and skin, or nebulization in broncho-pulmonar processes. Both these latter methods have given good results as employed by SANTOS SILVA (loc. cit.).
d) prophylaxis of possible sulphonamidotherapic accidents;
e) employ of a polivalent autogen vaccine;
f) disinfection of lesions of skin or mucous membrane with merthiolate or hexyl-resorcinol.

We think that the treatment of blastomycosis should be made by "assaults' the patient should follow the doctor's orders strictly, thus ensuring a complete success of the therapy. The treatment should be started as soon as possible.

To obtain control over the cure of blastomycosis one should perform the following examinations:

1) a careful clinical examination, especially of mouth, pharynx, skin and lungs;
2) a biopsy of any accessible lesion, followed by a histopathological examination, culture of material obtained, and inoculation into guinea pig's testicles;
3) hemosedimentation;
4) fixation of complement test;

The patients should visit the Service regularly, in order to verify the possibility of a relapse.

In the work of ALMEIDA, LACAZ and CARDOSO DA CUNHA (17) there are more minutiae to be found regarding treatment and cure control of South-American blastomycosis. The present results from experience we had in daily contact with pacients suffering with blastomycosis; it focuses in general lines a problem of big medico-social interest in Brazil. Studies on this infection have been made by a great many Brazilian workers, especially by the mycological school of São Paulo, led by FLORIANO DE ALMEIDA, whose works imparted a big impulse to better knowledge of blastomycosis.

Histological material, photographs and cultures of *Paracoccidioides brasiliensis* can be obtained with the A. of the present work at the following address: Faculdade Medicina Saõ Paulo. Estado de São Paulo. Brazil.

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The Use of Yeast Cells to Enhance the Virulence of *Candida albicans* for Mice [1] [2]

by

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(Plates XLIII-XLIV)

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Introduction

In a previous study (SCHERR, 1952) a number of compounds, including some auxins, were examined for their ability to enhance yeast phase to filament formation (Y → F) in *Saccharomyces cerevisiae*. It was speculated that auxins might be responsible for enhancing Y → F in yeasts and might serve to arrest multiplication of yeastlike pathogens *in vivo* for reasons which were discussed in the above paper.

The first step toward applying the auxin concept for Y → F formation, as previously propounded, in an attempt to effect a yeast to mycelial (Y → M) transition of the yeastlike fungi *in vivo*, was to test the effects of some of the plant growth substances on mice systemically infected with *Candida albicans*.

The strong M → Y activity which occurs when an M phase yeastlike fungus invades the body is well known. *C. albicans* examined directly from infected tissues appears as oval, budding, yeastlike cells. Occasionally mycelial fragments are found, especially with material taken from infected kidneys (BENHAM, 1931; HOPKINS, 1932). When mycelial growth occurs *in vivo* it usually does so late in the course of the disease and prior to fatal termination. Fig. 1 (a, b, c) shows representative fields demonstrating the morphology of *C. albicans* cells from lesions in the kidney, omentum, and spleen, respectively, of infected mice. Mycelial formation has not yet occurred, but some Y → F activity is represented by the "sprouting" yeast cells. Fig. 2, on the other hand, representing conditions similar to those above except that the infection has progressed 6 times as long as in the first case, shows a marked mycelial dissemination.

In the latter case, the possibility that the M → Y mechanism of the *Candida* cells has been affected seems remote, since M cells taken from an infected animal will revert to the Y phase at 37° C just as readily as M cells taken from a culture tube. It appears that some one or more of the bodily mechanisms responsible for M → Y transformation have broken down. Except in the later stages of the disease, one or more mechanisms must be in operation

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[1] A portion of this work has been presented before the 51st general meeting of the Society of American Bacteriologists in Chicago, Illinois, May, 1951 (SCHERR and WEAVER, 1951).

[2] This report is taken from a dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Kentucky, Lexington.

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in the animal which either prevent mycelial formation in the yeastlike pathogenic fungus or enhance its yeast phase formation. Although the body temperature (approximately 37° C) in infected animals no doubt plays a major role in the *in vivo* M → Y transformation, as evidenced by *in vitro* studies, that other factors are at work, aside from temperature, in affecting M → Y formation *in vivo* is indicated by the fact that some Y → M activity *is* found *in vivo*, at least for *Candida* infections. It is also significant in this respect that Y → M formation in a systemic infection has not been reported, at least to this writer's knowledge, for pathogens which display thermal dimorphism (*Blastomyces dermatitidis*, *Blastomyces brasiliensis*). Those pathogens which have nutritional as well as temperature requirements for yeast phase formation may be maintained in the M phase at 37° C by varying the contents of the culture medium. Thus SALVIN (1949) showed that *Histoplasma capsulatum* required a sulfur (S) or sulfhydryl (—SH) group, provided by a small organic molecule, plus biotin in order to maintain the yeast phase at 37° C; removing these required substances, while maintaining the incubation temperature of 37° C, caused the cells to enter the mycelial phase.

It would be presumptuous of the author to attempt a review of the characteristics of *Candida* in the light of DIDDENS and LODDER's excellent monograph (1942). Other pertinent data have been presented by FINEMAN (1921), BENHAM (1931), DODGE (1935), DE ALMEIDA (1939), MARTIN and JONES (1940), CONANT *et al.* (1944), and SKINNER *et al.* (1947).

Materials and Methods

The medium referred to as S.G. medium, information pertinent to the photographs, and the culture of *S. cerevisiae* used in this study have all been previously discussed (SCHERR, 1952).

The strain of *C. albicans* used in this work was procured from Dr. Margaret Hotchkiss of the Department of Bacteriology of the University of Kentucky. She had originally received this culture from Dr. Forest Spindle, of the Stuart Circle Hospital, Richmond, Va., who had isolated it from the lung pus of a patient.

A number of preliminary experiments were run at the outset of this investigation to determine the dose of *C. albicans* required to induce a systemic infection in mice. Five mice were intraperitoneally injected with 0.5 ml amounts of a washed cell suspension of yeast phase cells of this strain of *C. albicans* harvested from a 48 hour 30 ml S.G. broth culture by suspending the centrifuged cells in 10 ml buffered distilled water (BDW) (AMERICAN PUBLIC HEALTH ASS., 1948). Only one of the animals showed a marked dissemination of moniliasis after 10 days. The culture recovered from this animal was designated as *C. albicans* 171D. It retained its greater virulence as compared to the original stock strain even after numerous transfers on S.G. agar.

The mice used in this work were a Swiss strain maintained in the Department of Bacteriology of the University of Kentucky. All the animals received water and Purina chow pellets, under both normal and experimental conditions.

The animal work recorded in this paper lends itself to a t analysis very readily and this method was suggested by Dr. D. E. South of the Department of Mathematics of the University of Kentucky. What was done essentially was to examine the significance of the mean values for the severity of infection with *C. albicans* in a group of treated animals compared to the mean values for a group of infected untreated controls. The following formula was taken from HOEL (1947):

$$t = \frac{(\bar{x}-\bar{y})-(M_x-M_y)}{\sqrt{N_xS_x^2+N_yS_y^2}}\sqrt{\frac{N_xN_y(N_x+N_y-2)}{N_x+N_y}}$$

where $\bar{x}$ is the mean for group x
$\bar{y}$ is the mean for group y
N_x is the number of mice in group x
N_y is the number of mice in group y
S_x is the standard deviation from the mean for group x
S_y is the standard deviation from the mean for group y
S_x, S_y is computed as outlined by EZEKIAL (1950):

Standard deviation $= \sqrt{\frac{\Sigma d^2}{n}}$ where n is the number of mice

d is the difference between the individual degree of dissemination and mean for the group of mice in which the individual is a member.

In applying this formula the assumption is made that the means for groups x and y (M_x and M_y, respectively) are equal, which strikes the expression $M_x - M_y$ from the formula, since if M_x and M_y are equal $M_x - M_y$ is equal to zero. The application of the rest of the formula now tests the significance of the difference between the actual means for groups x and y. If $\bar{x}$ and $\bar{y}$ are actually equal, than t becomes O and the P (probability) value determined from t tables (RIDER, 1939) is 1. This would indicate that $\bar{x}$ will equal $\bar{y}$ in every case on a chance basis. As the value for t becomes greater, the P value becomes smaller, indicating that chance has played a small part in determining the differences between $\bar{x}$ and $\bar{y}$. P values are read from prepared tables, using the data for t and v (the number of degrees of freedom).

$$v = N_x + N_y - 2$$

If the treatment of a group of infected mice decreases the degree of dissemination, than t will be a positive integer; if treatment aggravates their condition, then the t value will be negative. P values greater than .05 are considered significant in this study; i.e., on the supposition that the mean values for dissemination are equal in two groups of mice, there are only 5 chances in 100 of observing a greater difference than that yielding a P value of .05.

Experimental

1. The effect of alpha-naphthalene acetic acid (ANAA) on mice systemically infected with *Candida albicans* 171D

a) Toxicity for normal mice.

Before testing the therapeutic efficacy of ANAA for infected animals, it was necessary to determine the toxicity of this compound for the mice to be used in this work. ANDERSON, SHIMKIN, and LEAKE (1936) had found that the toxic level of ANAA for mice was 100 mg/kg of body weight. In their work the animals were inoculated intraperitoneally with varying volumes of a 1 % dilution of the compound in di-ethylene glycol and its toxic levels were interpreted in terms of the MLD_{50}.

Since the type of infection in this work is of such a nature as to require periodic treatment over a relatively long period of time, the toxic level of ANAA for the mice was determined by the following procedure.

A stock solution of ANAA was made by adding 0.5 g of powdered ANAA (Kolker Chemical Co.) and 20 ml of 95 % ethanol to a 50 ml volumetric flask and diluting to the mark with distilled water. This stock solution of ANAA (0.01 g/ml) was serially diluted, using a mixture of 20 ml of 95 % ethanol and 30 ml water as a diluent, to yield 0.001, 0.0001, and 0.00001 g/ml concentrations of ANAA. No attempt was made to sterilize these solutions and they were kept in the refrigerator when not in use.

Groups of 5[1]) Swiss mice were used for each concentration of ANAA to be tested. Each of the mice was inoculated intraperitoneally with 0.2 ml of the respective dilution. These inoculations were repeated every 24 hours for a period of 5 days. At the end of the fifth day all surviving mice were weighed, sacrificed, and autopsied. The weights of mice that had died before the end of the experiment are not recorded in this or subsequent experiments. The results of this experiment are recorded in table 1.

TABLE 1. *Toxicity of alpha-naphthalene acetic acid (ANAA) for mice inoculated intraperitoneally*

(Duration of experiment — 5 days)

Dose (every 24 hrs.)	Mouse No.	Sex	Weight At start	Weight After 5 days	Death in days
	I	F	23.5	20.3	
	II	F	22.4		1
0.001 gm	III	M	22.8		1
	IV	M	25.9		1
	V	F	19.3		3
	I	F	22.8	24.2	
	II	M	26.1	25.9	
0.0002 gm	III	F	27.3	25.6	
	IV	F	21.3	23.9	
	V	F	24.9	24.0	
	II	M	28.3	27.3	
0.0002 gm	III	F	24.4	23.8	
	IV	M	29.0	31.1	
	V	F	20.0	20.0	
	I	M	26.3	26.5	
	II	M	21.9	21.7	
0.000002 gm	III	M	30.1	29.6	
	IV	M	29.6	29.9	
	V	M	28.0	28.1	

The relative weights of the animals at the beginning and end of the experiment are without significance.

A dose of 1 mg of ANAA was fatal for these animals, 3 out of 5 dying within 24 hours after inoculation; or, an average total of approximately 88 mg/kg of body weight, when given in 3 equal daily doses, was fatal to

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[1]) In this and all subsequent experiments, mention of any animal which died as the result of causes not pertinent to the experiment (faulty inoculation, fighting, etc.) was omitted from the tabulated results. Since each mouse was identified by a number at the start of each experiment, the omission of one or two animals in the data may leave a table of mouse numbers not arranged consecutively.

4 of 5 mice. This result is similar to that of ANDERSON, SHIMKIN, and LEAKE (1936).

In general, it was felt that it would be advisable to test those compounds for their therapeutic efficacy that showed toxic levels for mice of not less than 1000 mg/kg of body weight. It was also felt that this toxic level should, for each decigram of mouse body weight, be the amount required to show *in vitro* $Y \rightarrow F$ activity at levels non-toxic to yeast cells. The latter criterion was arrived at from the following considerations. The factor 10 was arrived at from considerations of the amount of binding of therapeutic agents in tissues and sera of animals. (See MACLEOD, 1949, for a more detailed discussion of this point.) The amount of binding that will occur is unpredictable, but the factor 10 indicated above appears to be conservative when it is noted, for example, that the concentrations of quinine and quinacrine in liver tissues are approximately 100 and 5000 times, respectively, the concentration in plasma (DAVIS, 1948).

The sub-toxic amount of ANAA required to elicit $Y \rightarrow F$ formation for *S. cerevisiae* in S.G. broth was 0.1 mg/ml (SCHERR, 1952). In order to gain approximately this level *in vivo*, approximately 10 times this amount would have to be injected into a mouse, to compensate for its dilution with the body fluids. Another factor of 10 should be considered for reasons of binding of foreign compounds by the body tissues and fluids. Therefore, 10 mg of ANAA would have to be inoculated into a mouse to approximate the concentration necessary for *in vitro* $\mathrm{Y} \rightarrow F$ activity. This value is equivalent to 500 mg/kg of body weight for a 20 g mouse and is approximately 6 times as great as the amount which proved to be fatal to 4 out of 5 mice. These calculations are submitted in order to indicate the standards used to decide what compounds to examine for their therapeutic efficacy.

It is obvious, from the above calculations, that the high toxicity of ANAA for mice makes it extremely unlikely that it could possibly have any *in vivo* $Y \rightarrow F$ activity at sub-toxic levels, allowing even for only a moderate amount of binding. Nevertheless, it was felt that it might be of interest to examine the effect of ANAA on the course of a systemic infection with *C. albicans*. The following experiment was performed for this purpose.

b) The effect of alpa-naphthalene acetic acid (ANAA) on infected mice

C. albicans 171D was loop-inoculated into 30 ml of S.G. broth contained in a 125 ml erlenmeyer flask. This culture was incubated at 30° C for 48 hours, after which time the cells were harvested by centrifugation, washed with BDW, and finally suspended in 15 ml of BDW. This final cell suspension contained 1.1×10^9 cells per ml as determined by plate count on S.G. agar.

Two sets of 5 mice each were inoculated intraperitoneally with 0.2 ml amounts of the *Candida* cell suspension. The mice of one group were set aside as infected controls. The mice of the other infected group and of a third group consisting of uninfected mice were inoculated intraperitoneally with 0.2 ml amounts of a 0.001 g/ml solution of ANAA prepared according to previous directions. The treatment with ANAA was repeated every 24 hours for a period of 5 days. This was followed by a 5 day rest period, and then the treatment was resumed for 5 more daily injections. At the end of the 15 day treatment period all surviving mice were weighed, sacrificed, and autopsied. Animals that died prior to the end of the experiment were autopsied immediately after being found dead or were refrigerated until autopsy could be performed.

In this and all subsequent mouse experiments the degree of dissemination of *Candida* was gauged from the number, size, and distribution of the lesions.

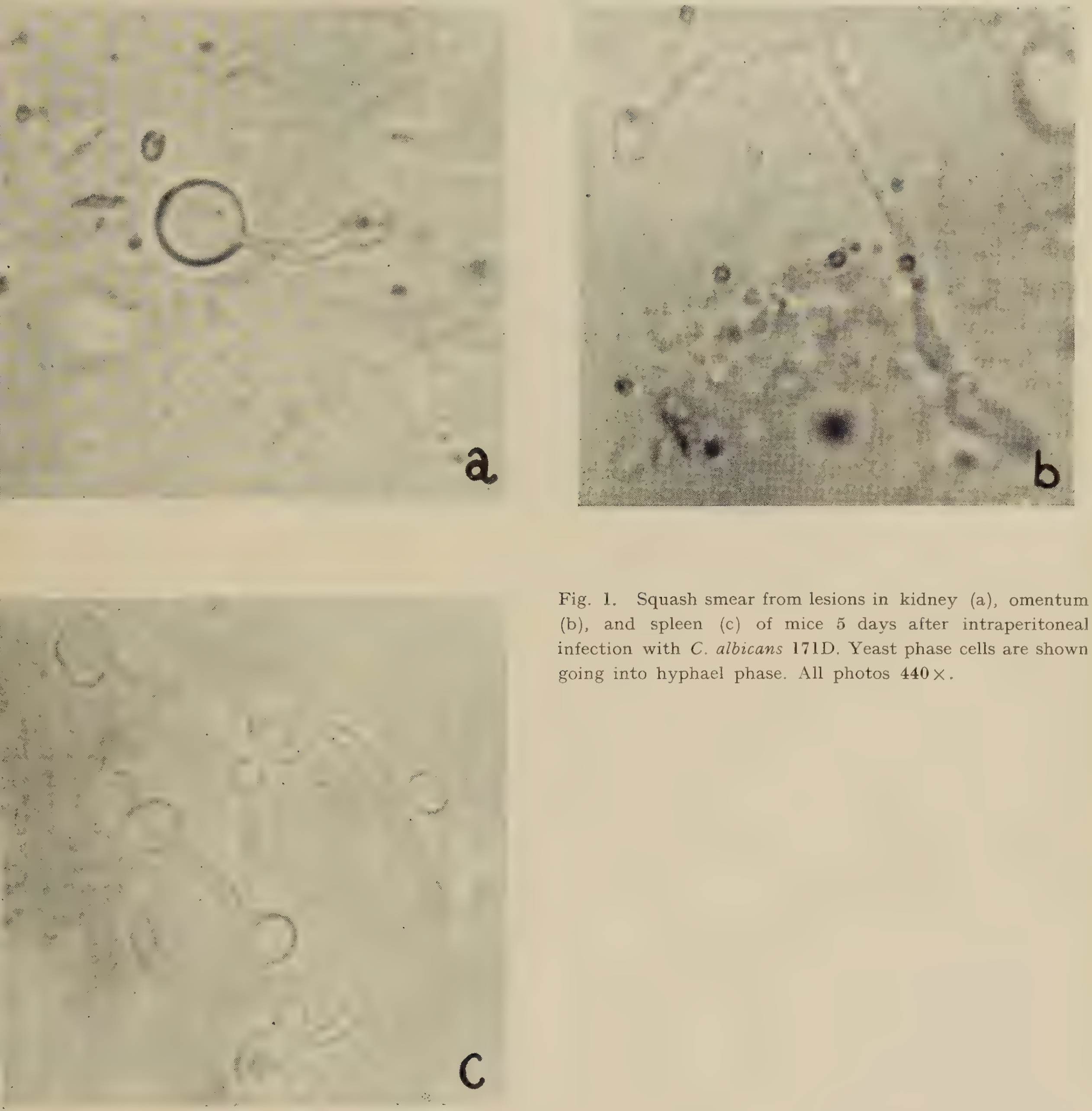


Fig. 1. Squash smear from lesions in kidney (a), omentum (b), and spleen (c) of mice 5 days after intraperitoneal infection with *C. albicans* 171D. Yeast phase cells are shown going into hyphael phase. All photos 440×.

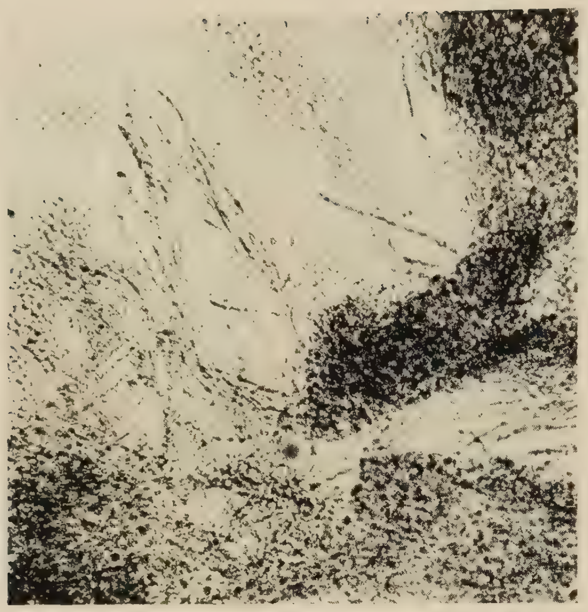


Fig. 2. Squash smear from lesion in kidney of mouse 30 days after intraperitoneal infection with *C. albicans* 171D. Mycelial phase of organisms predominates. 100×.

As a rule the condition of the kidneys is an excellent indicator of the severity of the infection. The degree of dissemination was assigned a numerical value, as follows:

0	no visible pathology
1	few, isolated lesions
2	frequent lesions
3	many lesions
4	marked dissemination of lesions
5	very marked dissemination

If the animal died, presumably as a result of *Candida* dissemination, prior to the end of the treatment period, a value 1 was added to the base value 5 for every day prior to the end of the experiment. For example, if an animal died 3 days before the experiment culminated, the severity of the infection was designated by a number 8.

Where statistical evaluation of the data was indicated, the method of *t* was employed.

Table 2 summarizes the data for this experiment. It is obvious at sight

TABLE 2. *The effect of alpha-naphthalene acetic acid (ANAA) on mice systemically infected with Candida albicans* 171D

(Duration of experiment — 15 days)

Legend	Mouse No.	Sex	Weight (gms) at start	Weight (gms) after 15 days	Dissemination	*d*	d^2
Infected, untreated	I	M	25.2	24.8	3	0.4	0.16
	II	M	29.7	29.4	4	0.6	0.36
	III	M	29.7	—	6	2.6	6.76
	IV	M	24.6	25.9	2	1.4	1.96
	V	M	26.7	27.6	2	1.4	1.96
	Total				17	6.4	11.20
	Mean				3.4		
	Average dev. from mean					1.28	
	Standard dev. from mean						1.5
Infected, treated (see text for schedule)	I	M	18.2	—	8	3.4	11.56
	II	M	28.8	—	18	6.6	43.56
	III	M	29.2	26.9	4	7.4	54.76
	IV	M	23.8	—	19	7.6	57.76
	V	M	26.4	—	8	3.4	11.56
	Total				57	28.4	179.20
	Mean				11.4		
	Average dev. from mean					5.7	
	Standard dev. from mean						5.98
	t				—2.26		
	Degrees of freedom				8		
	P				.025—.05		
Uninfected, treated as above	I	M	28.5	29.8	0		
	II	M	30.2	31.1	0		
	III	M	27.5	27.9	0		
	IV	M	12.0	17.8	0		
	V	M	26.5	23.5	0		

that treatment with ANAA did not reduce the severity of the infection with *C. albicans*. On the contrary, the probability (*P*) value between .025 and .05 is significant in indicating that the course of infection was aggravated by treatment. The reason for this is not evident.

The weight measurements were not significant, some of the animals gaining, others losing weight, both in the infected untreated and uninfected treated groups of mice.

Since the toxicities for mice of 3-indole acetic acid and indolepropionic acid are comparable to those of ANAA (25, 100, and 100 mg/kg of body weight, respectively; Anderson, Shimkin, and Leake, 1936), it was decided not to pursue the investigation with these compounds.

2. The effect of *Saccharomyces cerevisiae* on mice systemically infected with *Candida albicans* 171D.

a) The effect of *S. cerevisiae* cell extract.

Kogl and Kostermans (1934) isolated 3-indole acetic acid from yeast plasmolysate and showed that this compound represented a considerable part of the auxin acitivity of yeast. The auxin precursor in plant tissues occurs in the form of a stable inactive auxin-ester from which the auxin is liberated very slowly (Skoog and Thimann, 1940) and at low pH levels (Strugger, 1932, 1933, 1934) only the free acid form has growth promoting activity (Bonner, 1934).

It was felt that 3-indole acetic acid from yeast cells might be less toxic to an animal than the crystalline compound, at an equivalent concentration, because the degradation of the auxin precursor *in vivo* would result in the more or less gradual liberation of free acid. Such an effect, if it were to occur *in vivo*, could not be duplicated by more frequent inoculations of a smaller dosage of the crystalline 3-indole acetic acid except by using a constant drip method, which is impractical for mice. An experiment was therefore run to determine the effect of a yeast cell extract on *Candida* infected mice.

S. cerevisiae was inoculated into a 1-liter flask containing 250 ml of S.G. broth. The resultant culture was incubated at 25° C for 5 days, and the cells were harvested by centrifugation, suspended in 10 ml of cold (5° C) BDW, and ground by hand with a sterile pestle and mortar, using sterile sand. The supernatant liquid was harvested with a pipette, using 5 ml cold BDW washings of the sand. The washings and supernatant liquor were pooled in a 50 ml round-bottom centrifuge tube and centrifuged for 30 minutes at 2000 rpm to bring down the cell debris and fine sand particles. The final supernatant liquid, approximately 40 ml, was opalescent. It was transferred to a vaccine bottle and stored in a freezer when not in use.

The suspension of *Candida* cells used to infect the mice was prepared in the same manner as previously described.

Two groups of 10 mice each were inoculated intraperitoneally with 0.2 ml amounts of the *Candida* cell suspension. The mice of one of the infected groups and the uninfected mice of a control group were inoculated intraperitoneally with 0.2 ml amounts of the yeast cell extract every 24 hours for a period of 10 days. Fifteen days after inoculation the animals were sacrificed and examined for *Candida* dissemination. The results are compiled in table 3.

The treatment with the yeast fraction did not affect the course of the *Candida* infection. The mice that received injections of the yeast fraction only appeared to be unaffected by this treatment. The weight determinations were again without significance.

There are numerous reasons that could explain the negative results:

TABLE 3. *Effect of Saccharomyces cerevisiae cell extract on mice systemically infected with Candida albicans* 171D

(duration of experiment — 10 days)

Legend	Mouse No.	Sex	Weight (g)		Dissemination	*d*	d^2
			at start	after 15 days			
Infected, untreated	I	M	22.1	22.7	4	0.4	0.16
	II	M	23.7	23.3	3	1.4	1.96
	III	M	25.0	25.2	4	0.4	0.16
	IV	M	29.0	28.3	4	0.4	0.16
	V	M	22.4	23.2	3	1.4	1.96
	VI	M	20.3	20.8	5	0.6	0.36
	VII	M	23.7	23.9	5	0.6	0.36
	VIII	M	25.6	24.8	4	0.4	0.16
	IX	M	23.5	23.6	5	0.6	0.36
	X	M	21.8	—	7	2.6	6.76
	Total				44	8.8	12.40
	Mean				4.4		
	Average dev. from mean					0.88	
	Standard dev. from mean						1.1
Infected, treated daily with 0.2 ml of extract	I	M	20.9	21.0	5	0.7	0.49
	II	M	19.3	20.2	4	0.3	0.09
	III	M	23.6	23.1	4	0.3	0.09
	IV	M	24.2	24.7	5	0.7	0.49
	V	M	24.8	23.8	2	2.3	5.29
	VI	M	22.1	22.5	2	2.3	5.29
	VII	M	23.7	23.9	4	0.3	0.09
	VIII	M	22.8	—	9	4.7	22.09
	IX	M	21.2	21.0	3	1.3	1.69
	X	M	18.9	19.2	5	0.7	0.49
	Total				43	13.6	36.10
	Mean				4.3		
	Average dev. from mean					1.36	
	Standard dev. from mean						1.9
Uninfected, treated as above	I	M	27.7	27.9	0		
	II	M	25.3	25.8	0		
	III	M	24.9	24.7	0		
	IV	M	26.2	26.0	0		
	V	M	26.9	27.2	0		
	VI	M	21.0	21.6	0		
	VII	M	18.7	19.3	0		
	VIII	M	24.8	24.9	0		
	IX	M	23.3	23.6	0		
	X	M	22.9	22.8	0		

1. The concept of treating mycotic infections by the use of $Y \rightarrow F$ inducing agents is unprofitable.

2. Auxins are not good $Y \rightarrow F$ inducing agents. This reason would appear to have been eliminated by the *in vitro* studies.

3. The lack of an optimum concentration of auxin *in vivo*.

4. The suppression of the physiological activity of auxin *in vivo*. The determination of this is beyond the scope of this study.

5. The inactivation of the auxin within the yeast cells in the process of extraction. This has been found to be a major source of error by many plant physiologists (WENT and THIMANN, 1937, p. 65). ROBINSON and WOODSIDE (1937) reported that auxin activity declines progressively as the interval between extraction and testing increases even if the solution is kept at 5° C in the dark.

Because the validity of the postulated reasons 2, 3, 4, and 5 is short of experimental support at this time, the judgment of the therapy of the mycotic infections by the use of $Y \rightarrow F$ inducing agents does not necessarily rest with the success or failure of the *in vivo* use of auxins.

It was felt that the points brought up in (3) and (5) might be answered if a greater concentration of auxin derived from the yeast cells could be inoculated and if this auxin could be introduced into the animal without the necessity of an extraction procedure. This might ensue if whole yeast cells were inoculated and their breakdown occurred *in vivo*. This was tried in the next experiment.

b) The effect of *S. cerevisiae* cells

A suspension of *C. albicans* 171D cells in BDW was prepared as previously outlined. A suspension of *S. cerevisiae* cells was prepared by inoculating 250 ml of S.G. broth contained in a 1-liter erlenmeyer flask and incubating this culture at 30° C for 48 hours with shaking. Shaking was accomplished with a reciprocating shaker having 68 occillations per minute and a full stroke of 2½ inches. The *S. cerevisiae* cells were harvested by centrifugation and suspended in 50 ml of BDW. The count on the final *C. albicans* cell suspension was 1.0×10^9 cells per ml; the count on the *S. cerevisiae* cell suspension was 9.3×10^9 cells per ml. Mice from two groups of 10 mice each were inoculated with 0.2 ml amounts of the *Candida* cell suspension. After 24 hours 0.2 ml amounts of the *S. cerevisiae* cell suspension were inoculated intraperitoneally into the mice of one of the infected groups and into the uninfected mice of another group used as a control. This treatment was continued on the same schedule as that outlined in section 2a. The results of this experiment are recorded in table 4.

The weight determinations were without significance and were discontinued in subsequent experiments.

The inoculation of the yeast cells into infected mice, rather than alleviating the dissemination of the infection, seemed to aggravate it considerably. The marked dissemination of *C. albicans* and the increased fatalities found among the infected mice which were treated with yeast cells could not be attributed to any toxic materials present in the yeast suspension, since all the treated controls survived the treatment with practically no indication of the induction of a pathological condition. Before any significance could be attached to these results, it was desirable to attempt to extend and confirm them by another experiment. The effects of *S. cerevisiae* cells and of *S. cerevisiae* cell-free filtrate, alone and together, on *Candida*-infected mice were then tried.

TABLE 4. *Effect of Saccharomyces cerevisiae cells on mice systemically infected with Candida albicans* 171D

(Duration of experiment — 15 days)

Legend	Mouse No.	Sex	Weight (gms)		Dissemination	*d*	d^2
			at start	after 15 days			
Infected, untreated	I	M	24.1	25.6	4	1.7	2.90
	II	M	23.0	26.8	5	0.7	0.49
	III	M	28.0	31.5	3	2.7	7.30
	IV	M	26.1	23.5	5	0.7	0.49
	V	M	19.9	—	15	9.3	86.40
	VI	M	24.4	24.5	5	0.7	0.49
	VIII	M	23.1	25.1	4	1.7	2.90
	IX	M	24.5	24.9	5	0.7	0.49
	X	M	27.5	26.8	5	0.7	0.49
	Total				51	18.9	101.95
	Mean				5.7		
	Average dev. from mean					2.1	
	Standard dev. from mean						3.4
Infected, treated with *S. cerevisiae* cell suspension (see text for schedule)	I	M	26.4	—	5	6.3	39.69
	III	M	21.3	20.9	1	10.3	106.09
	IV	M	26.4	—	23	11.7	136.89
	VI	M	27.4	—	11	0.3	0.09
	VII	M	23.2	—	24	12.7	161.29
	VIII	M	27.9	27.5	1	10.3	106.09
	IX	M	17.4	—	13	1.7	2.89
	X	M	30.8	—	12	0.7	0.49
	Total				90	54.0	553.52
	Mean				11.3		
	Average dev. from mean					6.8	
	Standard dev. from mean						8.3
	t				—1.73		
	v				15		
	P				.05—.1		
Uninfected, treated as above	I	M	27.8	29.6	0		
	II	M	25.8	30.2	0		
	III	M	26.2	27.9	0		
	IV	M	22.5	23.3	0		
	V	M	24.3	25.7	0		
	VI	M	28.7	28.2	2[1]		
	VII	M	26.4	27.9	1[1]		
	VIII	M	16.1	18.5	0		
	IX	M	16.2	22.4	0		
	X	M	26.8	28.8	0		

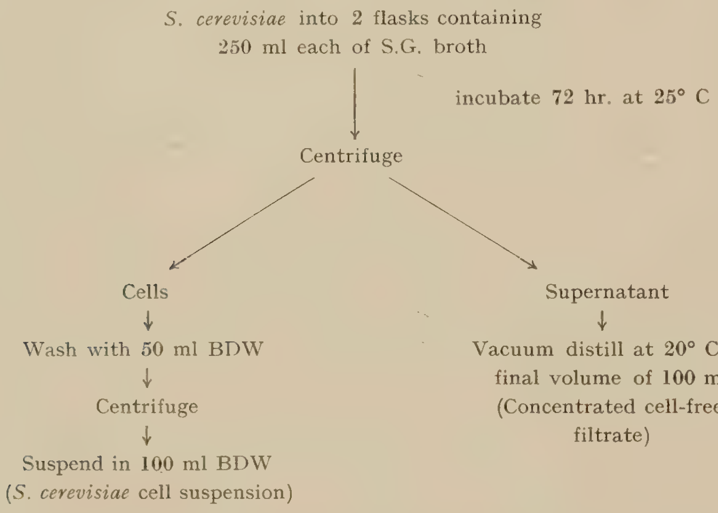
---

[1] Lesions were found at the site of inoculation, with slight dissemination to the mesentery. Organisms cultured from both animals proved to be *s. cerevisiae.*

c) The effect of *S. cerevisiae* cells plus/or *S. cerevisiae* cell filtrate.

C. albicans 171D was inoculated into 30 ml of S.G. broth contained in a 125 ml erlenmeyer flask and the resultant culture was then incubated at 25° C. After 48 hours of incubation the *Candida* cells were harvested by centrifugation and suspended in 16 ml of BDW. Four groups of 10 mice each were inoculated intraperitoneally with 0.4 ml amounts of this *Candida* cell suspension.

In the preparation of the *S. cerevisiae* cell suspension and cell-free filtrate 2 1-liter erlenmeyer flasks containing 250 ml of S.G. broth were inoculated and incubated at 25° C for 72 hours. The cells were harvested by centrifugation from the pooled cultures, washed, and suspended in 100 ml of BDW. The remaining filtrate was vacuum distilled at 20° C to reduce it to a volume of 100 ml. The procedure is outlined in the following scheme:


S. cerevisiae into 2 flasks containing
250 ml each of S.G. broth
incubate 72 hr. at 25° C
Centrifuge
Cells
Wash with 50 ml BDW
Centrifuge
Suspend in 100 ml BDW
(*S. cerevisiae* cell suspension)
Supernatant
Vacuum distill at 20° C to
final volume of 100 ml
(Concentrated cell-free
filtrate)

In this experiment the following groups of 10 mice each were used: infected, untreated; infected, treated with *S. cerevisiae* cells; infected, treated with concentrated *S. cerevisiae* cell-free filtrate; infected, treated with cells plus filtrate; uninfected, treated with cells; uninfected, treated with filtrate; uninfected, treated with cells plus filtrate. Treatment, consisting of the intraperitoneal injection of 0.2 ml amounts of the cells or filtrate, was commenced 24 hours after injection with the *Candida* cells. Animals treated either with the cells or with the filtrate received an inoculation every 48 hours; those mice receiving the cells plus the filtrate received alternate injections of cells and filtrate at 48-hour intervals. This schedule of treatment was interrupted following the 6th and 15th days after infection by 5-day rest periods, during which time no injections were made. Twenty days after infection all surviving animals were autopsied. The results are compiled in table 5.

The data for pregnant mice are not included in the computations for the mean values for dissemination. Work to be described later has indicated that pregnancy distinguishes the infected animal as a special case; consequently, results with pregnant animals cannot be evaluated with those with non-pregnant animals.

This experiment confirmed the data of the previous one in showing that *Candida* infected mice suffer an increased morbidity, compared to infected

TABLE 5. *The effect of Saccharomyces cerevisiae cells plus/or Saccharomyces cerevisiae cell filtrate on mice systemically infected with Candida albicans* 171D

(Duration of experiment — 20 days)

Legend	Mouse No.	Sex	Dissemination	d	d^2
Infected, untreated	I	F	5	1.6	2.56
	II	F	17	10.4	108.00
	III	M	4	2.6	6.76
	IV	F	5	1.6	2.56
	V	M	5	1.6	2.56
	VI	F	2 (pregnant)		
	VII	M	5	1.6	2.56
	VIII	F	3 (pregnant)		
	IX	F	2 (pregnant)		
	X	M	5	1.6	2.56
	Total		46	21.0	127.56
	Mean		6.6		
	Average dev. from mean			3.0	
	Standard dev. from mean				4.3
Infected, treated with *S. cerevisiae* cells	I	F	19	0.8	0.64
	II	F	19	0.8	0.64
	III	M	24	4.2	17.64
	IV	F	24	4.2	17.64
	V	F	24	4.2	17.64
	VI	F	17	2.8	7.84
	VII	F	10	9.8	96.00
	VIII	F	21	1.2	1.44
	IX	F	19	0.8	0.64
	X	M	21	1.2	1.44
	Total		198	30.0	161.56
	Mean		19.8		
	Average dev. from mean			3.0	
	Standard dev. from mean				4.0
	t		—6.16		
	Degrees of freedom		15		
	P		∠.005		

Table 5 (continued).

Legend	Mouse No.	Sex	Dissemination	d	d^2
Infected, treated with *S. cerevisiae* cell-free filtrate	I	F	4	1.3	1.69
	II	F	3	2.3	5.29
	III	F	2	3.3	10.90
	IV	M	14	8.7	75.69
	V	M	5	0.3	0.09
	VI	F	2 (pregnant)		
	VII	M	5	0.3	0.09
	VIII	F	5	0.3	0.09
	IX	F	5	0.3	0.09
	X	M	5	0.3	0.09
	Total		48	17.1	94.02
	Mean		5.3		
	Average dev. from mean			1.9	
	Standard dev. from mean				3.2
	t		.65		
	Degrees of freedom		14		
	P		.25—.3		
Infected, treated with cells plus filtrate	I	F	2 (pregnant)		
	II	F	21	4.0	16.00
	III	F	19	2.0	4.00
	IV	F	18	1.0	1.00
	V	M	10	7.0	49.00
	VI	F	24	7.0	49.00
	VII	F	11	6.0	36.00
	VIII	M	20	3.0	9.00
	IX	F	19	2.0	4.00
	X	M	11	6.0	36.00
	Total		153	38.0	204.00
	Mean		17		
	Average dev. from mean			4.2	
	Standard dev. from mean				4.8
	t		—4.19		
	Degrees of freedom		14		
	P		∠.005		

Table 5 (continued).

Legend	Mouse No.	Sex	Dissemination
Uninfected, treated with cells	I	M	0
	II	M	0
	III	F	0
	IV	F	0 (pregnant)
	V	F	0 (pregnant)
	VI	F	0
	VII	F	0
	VIII	F	0 (pregnant)
	IX	F	0
	X	F	0
Uninfected, treated with cell filtrate	I	F	0 (pregnant)
	II	M	0
	III	M	0
	IV	M	0
	V	M	0
	VI	F	0
	VII	M	0
	VIII	F	0
	IX	M	0
	X	F	0 (pregnant)
Uninfected, treated with cells plus filtrate	I	F	1 (small lesion on kidney)
	II	F	0
	III	F	0 (pregnant)
	IV	F	0
	V	F	0
	VI	F	0 (pregnant)
	VII	F	0
	VIII	M	0
	IX	M	0
	X	M	0

controls, when inoculated periodically with yeast cells. The use of *S. cerevisiae* filtrate had no effect on the course of the infection. The administration of cells and cell-filtrate together gave the same response as the cells alone. The probability (*P*) value for the infected group treated with yeast cells, computed using the infected untreated group as a standard, is small enough (∠ .005) to leave little doubt as to the statistical validity of the difference in degrees of *Candida* dissemination between the two groups of mice. This same correlation is applicable to the infected mice treated with cells plus filtrate.

The uninfected animals that received the same series of injections as the infected ones showed no pathological conditions at autopsy; a small lesion on the kidney of one of the mice did not contain a yeast. No further attempt at identification of the causative organism was made.

At this point an attempt was made to list possible explanations of the observed phenomenon, abandoning for the time being the pursuit of the original thesis.

Anaphylactic shock was considered as an unlikely explanation, since none of the uninfected mice died or showed any other symptoms of shock, though they received the same series of yeast cell and filtrate inoculations as the infected group. The production of anaphylaxis using yeasts is possible, however, and has been reported by ROSENAU and ANDERSON (1909).

The possibility was considered to exist that the effect of increased morbidity might be due to a transformation of the yeast cells to some virulent type, in a manner comparable to that reported for the pneumococcus (GRIFFITH, 1928), colon bacillus (BOIVIN, 1947), and other organisms.

It was also considered that the role of the yeast cells might be to enhance the virulence of the *Candida* cells in a manner similar to that of mucin (MILLER, 1933; MILLER and CASTLES, 1936; COHEN, 1936; and numerous other workers). The exaltation of pathogenicity produced by mucin and other substances with similar activity (agar, starch, gelatin) appears to be due to some action on the defense mechanisms of the body (NUNGESTER et al., 1936; MILLER and CASTLES, 1936; and KEEFER and SPINK, 1938).

Another possibility that was considered was the induction in the infected mice by the yeast inocula of a condition which FELTON and his co-workers call "immunological paralysis" (FELTON and OTTINGER, 1942; FELTON et al., 1947).

A further possibility was considered to be the induction of a state of "hypersensitivity" in a manner comparable to that described by NAKAYAMA (1906) and MATHIESON et al. (1935) for actinomycete infections.

These possibilities will be discussed subsequently in greater detail.

In order to determine the significance of the time of inoculation of yeast cells in relation to the time of infection with *C. albicans* in order to elicit this increased morbidity in infected mice, and the quantity of yeast cells required, the following series of experiments was performed.

d) The effect of a single small dose of *S. cerevisiae* cells.

In this experiment the infected mice were inoculated with a single dose of yeast cells comparable in size to the single doses given in the previous experiments. At the same time it was felt desirable to ascertain if the mice infected with *Candida* cells which had been grown in a medium containing mouse blood would show a more marked dissemination of moniliasis than mice infected with *Candida* cells grown in S.G. broth alone. Accordingly, this experiment was run in duplicate, one set of animals receiving *Candida* cells grown in S.G. broth containing 1 ml of whole mouse blood (heart's blood from right ventricle) per 30 ml of medium. The *S. cerevisiae* and *Candida* cells were prepared in the manner previously described.

All the animals were infected by injecting 0.4 ml amounts of the prepared *Candida* suspensions intraperitoneally. The mice in each group of 10 received single intraperitoneal injections of 0.2 ml of the yeast cell suspension according to the following schedule: one group, 24 hours prior to infection; one group, concomitantly with infection; and the other groups, 24, 48, 72, 96, 120, and 144 hours after infection. One group of infected mice remained uninoculated with yeast cells as a control group. Since the absence of a pathological condition in mice receiving yeast cell inoculations only had already been demonstrated, no control group of uninfected treated mice was required. Fifteen days after infection, all the surviving animals were sacrificed and autopsied. The results are compiled in table 6.

The *Candida* cells grown in S.G. broth containing blood did not cause any greater severity of infection than those grown in S.G. broth alone. The mean

TABLE 6. *The effect of a single small dose of Saccharomyces cerevisiae cells on mice systemically infected with Candida albicans* 171D

(Duration of experiment — 15 days)

		Candida cells grown in S.G. broth		*Candida* cells grown in S.G. broth containing mouse blood	
Legend	Mouse No.	Sex	Dissemination	Sex	Dissemination
Infected controls	I	F	4	M	5
	II	F	1 (preg.)	M	5
	III	F	2	F	2
	IV	M	4	F	10
	V	M	4	M	3
	VI	M	4	M	5
	VII	F	1 (preg.)	M	4
	VIII	F	9	F	1 (preg.)
	IX	M	8	M	3
	X	M	5	M	4
	Mean		5.0		4.6
Yeast cells inoculated 24 hrs. prior to infection	I	M	1	F	3 (preg.)
	II	F	19	F	1 (preg.)
	III	M	18	M	2
	IV	M	1	M	1
	V	F	1	M	7
	VI	F	1	M	1
	VII	M	4	F	3
	VIII	M	1	M	2
	IX	M	1	M	1
	X	M	2	F	1
	Mean		4.9		2.3
Yeast cells inoculated at same time as infection	I	F	0 (preg.)	F	0
	II	F	2	F	1 (preg.)
	III	M	6	F	1
	IV			F	2
	V			M	2
	VI	F	2	M	2
	VII	F	17	F	0 (preg.)
	VIII	F	17	M	3
	IX	M	15	F	3 (preg.)
	X	F	1	F	0
	Mean		8.6		1.4

Table 6 (continued).

		Candida cells grown in S.G. broth		*Candida* cells grown in S.G. broth containing mouse blood	
Legend	Mouse No.	Sex	Dissemination	Sex	Dissemination
Yeast cells inoculated 24 hrs. after infection	I	M	3	F	2
	II	F	2 (preg.)	F	1
	III	F	1	F	0 (preg.)
	IV			M	2
	V	M	4	F	2
	VI	F	3 (preg.)	F	2
	VII	F	4	M	3
	VIII	M	2	F	4
	IX	F	4	F	1
	X	F	3	M	3
	Mean		3.0		2.2
Yeast cells inoculated 48 hrs. after infection	I	M	5	F	2
	II	F	1	F	4
	III	F	2 (preg.)	F	0
	IV	M	3	M	18
	V	F	13	F	12
	VI	F	3 (preg.)	F	0
	VII	M	4	F	2
	VIII	F	3	F	2
	IX	M	14	F	1
	X	F	3	F	3
	Mean		5.8		4.4
Yeast cells inoculated 72 hrs. after infection	I	M	3	F	1 (preg.)
	II			M	2
	III	F	3	F	1
	IV	M	2	F	4
	V	F	4	F	5
	VI	F	5	M	2
	VII	F	3	M	2
	VIII	F	1	F	2
	IX	M	3	M	2
	X	M	3	M	2
	Mean		3.0		2.3

Table 6 (continued).

		Candida cells grown in S.G. broth		*Candida* cells grown in S.G. broth containing mouse blood	
Legend	Mouse No.	Sex	Dissemination	Sex	Dissemination
Yeast cells inoculated 96 hrs. after infection	I	M	2	M	2
	II	M	2	F	2
	III	M	2	M	3
	IV	F	2 (preg.)	M	2
	V	F	1 (preg.)	F	4
	VI	F	3	M	2
	VII	F	1 (preg.)	F	0 (preg.)
	VIII	F	10	F	1
	IX	M	3	F	0 (preg.)
	X	F	3	F	5
	Mean		3.6		2.6
Yeast cells inoculated 120 hrs. after infection	I	M	2	F	0 (preg.)
	II	F	3	F	10
	III	F	2	M	4
	IV	F	1 (preg.)	M	1
	V	M	3	F	1
	VI	M	3	F	2
	VII	F	1	M	3
	VIII	M	2	M	1
	IX	F	2	F	9
	X	M	1	F	0 (preg.)
	Mean		2.1		3.9
Yeast cells inoculated 144 hrs. after infection	I	M	3	F	1
	II	M	2	F	3
	III	F	2	M	2
	IV	M	3	M	2
	V	F	2	M	3
	VI	F	1	M	2
	VII	F	3	F	8
	VIII	F	3	M	3
	IX	M	3	F	2
	X	M	2	M	1
	Mean		2.4		2.7

values for the degrees of dissemination for all the groups of mice fell fairly well within narrow limits and no statistical evaluation seemed necessary. The course of the infection was not modified to any significant degree by the yeast cells. The mean value of 8.6 for the group of mice inoculated at the same time as the infection was higher than other values obtained; the reason for this is not clear.

It appears that more yeast cells than were contained in the single dose given in this experiment are required to enhance moniliasis in mice. To ascertain if the enhanced dissemination found in the previous experiment was due to the periodicity of yeast cell inoculations or to the sum total of yeast cells inoculated, the following experiment was run.

e) The effect of a single large dose of *S. cerevisiae* cells.

In a previous experiment (section *c*) mice treated with a suspension of *S. cerevisiae* cells received a total of 10 inoculations of 0.2 ml each. It was desired to prepare a suspension of *S. cerevisiae* cells so that a single inoculum of 0.2 ml would be equivalent to the sum total of cells of the 10 inoculations in the previous experiment. This was done as follows.

Four 1-liter flasks, each containing 250 ml of S.G. broth, were inoculated with *S. cerevisiae* and incubated at 25° C for 72 hours. At the end of this incubation period the cells were harvested by centrifugation, pooled, washed with BDW, and suspended in 40 ml of BDW. This constituted the concentrated cell suspension of *S. cerevisiae*.

If the single dose of the heavy yeast suspension were to modify the course of dissemination of *C. albicans* in mice, then it would be desirable to ascertain the relationship of the time of inoculation to the effect produced. This experiment was therefore planned so that mice would receive the single 0.2 ml inoculum of yeast cells intraperitoneally 24 hours prior to, concomitantly with, and 24, 48, and 72 hours after the infection with *C. albicans*. The mice were infected with a single dose of *C. albicans* 171D cells in the same manner as was done in the last experiment. A control group of uninfected mice received only the yeast inoculum. All surviving animals were sacrificed and autopsied 20 days after being infected with *C. albicans*. The results of this experiment are outlined in table 7.

Animals that received the yeast cell inoculum 24 hours before infection showed a comparable degree of dissemination to that of the infected controls. Statistical evaluation of the data is in accord with this conclusion, the P value between .15 and .1 indicating that the difference between the mean dissemination values for the treated and untreated groups is without significance. This is in striking contrast to the results for the groups of mice inoculated with the yeast cells at the same time and at different intervals after infection with *Candida*. All these groups showed a markedly increased dissemination of the infection and a increased death rate. Note the very high dissemination values of these groups as compared to the infected control group. The low *P* values indicate that it is extremely unlikely that the results could be due to chance. There is some suggestion that the delayed injection of yeast cells at the 48 and 72 hour intervals elicited a lessened degree of dissemination as compared to that elicited by the concomitant and the 24 hour interval injections. This is indicated by the trend of mean dissemination values for mice receiving the yeast cell inoculum 0, 24, 48, and 72 hours after infection, which are 11.9, 12.7, 7.8, and 7.3, respectively. The resolution of this point would require further experiments.

Benham (1935) has shown that *S. cerevisiae* is related antigenically to

TABLE 7. *The effect of a single large dose of Saccharomyces cerevisiae cells on mice systemically infected with Candida albicacs* 171D

(Duration of experiment — 20 days)

Legend	Mouse No.	Sex	Dissemination	d	d^2
Infected controls	I	F	1	2.0	4.00
	II	F	0 (pregnant)		
	III	F	3	0.0	0.00
	V	F	5	2.0	4.00
	VI	F	4	1.0	1.00
	VII	M	4	1.0	1.00
	VIII	M	3	0.0	0.00
	IX	F	3	0.0	0.00
	X	F	1	2.0	4.00
	Total		24	8.0	14.00

Mean 3.0
Average dev. from mean 1.0
Standard dev. from mean 1.3

Legend	Mouse No.	Sex	Dissemination	d	d^2
Yeast cells inoculated. . . . . 24 hours prior to infection	I	M	5	0.9	0.81
	II	M	5	0.9	0.81
	III	M	2	2.1	4.41
	IV	F	6	1.9	3.61
	V	F	4	0.1	0.01
	VI	F	5	0.9	0.81
	VII	M	8	3.9	15.21
	VIII	M	1	3.1	9.61
	IX	M	3	1.1	1.21
	X	F	2	2.1	4.41
	Total		41	17.0	40.90

Mean 4.1
Average dev. from mean 1.7
Standard dev. from mean 2.0

t . . . . —1.27
Degrees of freedom 16
P . . . . .15—.1

Table 7 (continued).

Legend	Mouse No.	Sex	Dissemination	d	d^2
Yeast cell inoculum, uninfected (control of above)	I	M	0		
	II	M	0		
	III	M	0		
	IV	F	0		
	V	M	0		
	VI	F	0		
	VII	M	0		
	VIII	F	0		
	IX	M	0		
	X	M	0		
Yeast cells inoculated at same time as infection	I	M	5	6.9	47.61
	II	F	16	4.1	16.81
	III	F	24	12.1	146.41
	IV	F	20	8.1	65.61
	V	F	3	8.9	79.21
	VI	M	19	7.1	50.41
	VII	M	18	6.1	37.21
	VIII	F	5	6.9	47.61
	IX	M	4	7.9	62.41
	X	M	5	6.9	47.61
	Total		119	75.0	600.90

Mean 11.9
Average dev. from mean 7.5
Standard dev. from mean 7.8

t for comparison to infected control . . . . —3.0
Degrees of freedom 16
P . . . . ∠.005

t for comparison to inoculum 24 hours prior to infection 2.9
Degrees of freedom 18
P . . . . ∠.005

Table 7 (continued).

Legend	Mouse No.	Sex	Dissemination	d	d^2
Yeast cell inoculum, uninfected (control of above)	I	M	0		
	II	M	0		
	III	M	0		
	IV	F	0		
	V	F	0		
	VI	M	0		
	VII	M	0		
	VIII	F	0		
	IX	M	0		
	X	M	0		
Yeast cells inoculated 24 hours after infection	I	M	5	7.7	59.29
	II	M	23	10.3	106.09
	III	F	23	10.3	106.09
	IV	F	21	8.3	68.8
	V	M	5	7.7	59.29
	VI	F	3	9.7	94.09
	VII	F	5	7.7	59.29
	VIII	M	19	6.3	39.69
	IX	F	5	7.7	59.29
	X	M	18	5.3	28.09
	Total		127	81.0	680.10

Mean 12.7
Average dev. from mean 8.1
Standard dev. from mean 8.2

t . . . . —3.1
Degrees of freedom 16
P . . . . ∠.005

Table 7 (continued).

Legend	Mouse No.	Sex	Dissemination	d	d^2
Yeast cell inoculum, uninfected (control of above)	I	M	0		
	II	M	0		
	III	F	0		
	IV	F	0 (pregnant)		
	V	F	0		
	VI	M	0		
	VII	M	0		
	VIII	M	0		
	IX	F	0		
	X	M	0		
Yeast cells inoculated 48 hours after infection	I	M	3	4.8	23.04
	II	M	5	2.8	7.84
	III	M	16	8.2	67.24
	IV	F	4	3.8	14.44
	V	F	9	1.2	1.44
	VI	M	15	7.2	51.84
	VII	M	5	2.8	7.84
	VIII	F	12	4.2	17.64
	IX	M	4	3.8	14.44
	X	M	5	2.8	7.84
	Total		78	41.6	213.60

Mean	7.8
Average dev. from mean	4.2
Standard dev. from mean	4.6
t	—2.69
Degrees of freedom	16
P	.005—.01

Table 7 (continued).

Legend	Mouse No.	Sex	Dissemination	d	d^2
Yeast cell inoculum, uninfected (control of above)	I	M	0		
	II	M	0		
	III	M	0		
	IV	F	0		
	V	M	0		
	VI	M	0		
	VII	F	0		
	VIII	M	0		
	IX	M	0		
	X	F	0		
Yeast cells inoculated 72 hours after infection	I	M	5	2.3	5.29
	II	M	18	10.7	114.49
	III	F	3	4.3	18.49
	IV	F	2	5.3	28.09
	V	M	13	5.7	32.49
	VI	M	4	3.3	10.89
	VII	M	9	1.7	2.89
	VIII	F	9	1.7	2.89
	IX	M	5	2.3	5.29
	X	M	5	2.3	5.29
Total			73	39.6	226.10

Mean 7.3
Average dev. from mean 3.96
Standard dev. from mean 4.8

t . . . . —2.3
Degrees of freedom 16
P . . . . .01—.025

Legend	Mouse No.	Sex	Dissemination
Yeast cell inoculum, uninfected, (control of above)	I	M	0
	II	M	0
	III	M	0
	IV	M	0
	V	M	0
	VI	F	0
	VII	F	0 (pregnant)
	VIII	M	0
	IX	M	0
	X	M	0

C. albicans. If this enhanced dissemination of moniliasis were associated in some way with this antigenic relationship, then it would be expected that a repetition of this experiment using yeast phase cells of *C. albicans* instead of *S. cerevisiae* would elicit the same response in infected mice. This approach is complicated, however, by the fact that *Candida* cells are pathogenic, so that their injection might mask any effect that would occur for reasons other than the pathogenicitiy of the *Candida* cells. It was thought, therefore, that formalin-killed yeast cells of *C. albicans* 171D might be employed to avoid this effect. Before this could be tried it was necessary to determine if formalin-killed *S. cerevisiae* cells would have the same effect as live cells.

f) The effect of formalinized cells of *S. cerevisiae.*

The suspension of *Candida* cells used to infect the mice was prepared as previously described. The formalinized yeast cells were prepared by the following procedure.

S. cerevisiae was inoculated into two 1-liter erlenmeyer flasks each containing 250 ml of S.G. broth and the resultant cultures were incubated at 30° C for 48 hours. The cultures were then centrifuged, the supernatant fluid was discarded, and the cell pellets were combined and suspended in approximately 50 ml of BDW. This cell suspension was again centrifuged, the supernatant fluid was discarded, and the cells were suspended in 100 ml of BDW containing 0.5 % formalin. It will be noted that this preparation of yeast cells differs from that employed in the experiment the results of which are outlined in section *c* only in being formalinized and in that the cells were from cultures that had been incubated without shaking. The formalinized yeast cells remained at room temperature for 48 hours, at which time a sterility test showed that the cells had been killed.

Two groups of 10 mice each were infected by intraperitoneal inoculations of 0.4 ml amounts of the viable *Candida* cell suspension. After 24 hours 0.2 ml amounts of the formalinized yeast cells were inoculated intraperitoneally into the mice of one of the infected groups and into the mice of an uninfected group serving as a control. The inoculation was repeated every 48 hours for 20 days, except for a 5 day rest period after the 6th day. At the end of 20 days all surviving animals were sacrificed and autopsied. The data are recorded in table 8.

The degree of dissemination enhanced by the formalinized yeast cells was equivalent to that found when living yeast cells were employed. These data are valid statistically and help to exclude transformation as a possible explanation for this phenomenon of enhanced virulence of *C. albicans.* The uninfected mice receiving the formalinized yeast cells were apparently unaffected by this treatment.

g) The effect of formalinized yeast-phase cells of *C. albicans* 171D.

The method used here was identical to that employed in the previous experiment except that formalinized *Candida* cells were substituted for formalinized yeast cells. The results are compiled in table 9.

The effect of the inoculations of formalinized yeast-phase cells of *C. albicans* 171D was to enhance the dissemination of *Candida* in infected mice. Animals receiving only the formalinized cell inoculations were apparently unaffected by this treatment.

It appears that some factor or factors responsible for increasing the morbidity in *Candida* infected mice is present in both *S. cerevisiae* and C. *albicans* 171D.

TABLE 8. *The effect of formalinized cells of Saccharomyces cerevisiae on mice systemically infected with Candida albicans* 171D

(Duration of experiment — 20 days)

Legend	Mouse No.	Sex	Dissemination	d	d^2
Infected, untreated	I	F	1	2.0	4.00
	II	F	0 (pregnant)		
	III	F	3	0.0	0.00
	V	F	5	2.0	4.00
	VI	F	4	1.0	1.00
	VII	M	4	1.0	1.00
	VIII	M	3	0.0	0.00
	IX	F	3	0.0	0.00
	X	F	1	2.0	4.00
	Total		24	8.0	14.00
	Mean		3.0		
	Average dev. from mean			1.0	
	Standard dev. from mean				1.3
Infected, treated with formalinized cells of *S. cereviciae* (see text for schedule)	I	M	16	6.2	38.44
	II	F	5	4.8	23.04
	III	M	21	11.2	125.44
	IV	M	14	4.2	17.64
	V	M	10	0.2	0.04
	VI	M	8	1.8	3.24
	VII	F	1 (pregnant)		
	VIII	F	3	6.8	46.24
	IX	F	7	2.8	7.84
	X	M	4	5.8	33.64
	Total		88	43.8	295.56
	Mean		9.8		
	Average dev. from mean			4.9	
	Standard dev. from mean				5.7
	t		—3.1		
	Degrees of freedom		15		
	P		0.01—0.001		
Uninfected, treated as above	I	M	0		
	II	F	0		
	III	M	0		
	IV	M	0		
	V	M	0		
	VI	M	0		
	VII	M	0		
	VIII	M	0		
	IX	F	0		
	X	F	0		

TABLE 9. *The effect of formalinized yeast-phase cells of Candida albicans* 171D *on mice systemically infected with Candida albicans* 171D

(Duration of experiment — 20 days)

Legend	Mouse No.	Sex	Dissemination	d	d^2
Infected, untreated	I	F	1	2.0	4.00
	II	F	0 (pregnant)		
	III	F	3	0.0	0.00
	V	F	5	2.0	4.00
	VI	F	4	1.0	1.00
	VII	M	4	1.0	1.00
	VIII	M	3	0.0	0.00
	IX	F	3	0.0	0.00
	X	F	1	2.0	4.00
	Total		24	8.0	14.00

Mean 3.0
Average dev. from mean 1.0
Standard dev. from mean 1.3

Legend	Mouse No.	Sex	Dissemination	d	d^2
Infected, treated with formalinized yeast-phase cells of *C. albicans* (see text for schedule)	I	M	22	9.5	90.25
	II	F	22	9.5	90.25
	III	M	4	8.5	72.25
	IV	M	12	0.5	0.25
	V	F	8	4.5	20.25
	VI	M	18	5.5	30.25
	VII	M	18	5.5	30.25
	VIII	F	5	7.5	56.25
	IX	F	5	7.5	56.25
	X	M	11	1.5	2.25
	Total		125	60.0	448.50

Mean 12.5
Average dev. from mean 6.0
Standard dev. from mean 6.7

t . . . . —3.71
Degrees of freedom 16
P . . . . $\angle .005$

Legend	Mouse No.	Sex	Dissemination	d	d^2
Uninfected, treated as above	I	M	0		
	II	F	0		
	III	M	0		
	IV	M	0		
	V	M	0		
	VI	F	0		
	VII	M	0		
	VIII	M	0		
	IX	M	0		
	X	M	0		

Summary and Discussion

It is clear from the data presented that some factor (or factors) is present in cells of *S. cerevisiae* and yeast-phase cells of *C. albicans* 171D which is responsible for enhancing the dissemination of systemic moniliasis in mice when they are inoculated intraperitoneally in sufficient dosage. This factor is not destroyed by treatment with 0.5 % formalin. It has not been possible to demonstrate its activity aside from the intact cell. Thus, a yeast cell extract did not affect the course of a *Candida* infection in mice.

None of the theories that have been postulated to explain this phenomenon appears to be entirely supported by the experimental data obtained. It has not been possible to obtain any evidence that the *S. cerevisiae* cells are transformed into a virulent type by contact with *Candida albicans* or its products.

The possibility that the function of the yeast cells and yeast-phase cells of *Candida* in enhancing the infection in mice could be attributed to an activity similar to that of mucin has not been disproven by the work reported here.

It has been interesting to consider the marked dissemination and increased death rate among infected mice which received yeast cell inoculations in relation to the observations of FELTON and his co-workers (FELTON and OTTINGER, 1942; FELTON et al., 1947; FELTON, 1949), who found that the injection of relatively large doses of the pneumococcus polysaccharide into mice so altered the immunological system that antibody could no longer be produced by a subsequent immunizing dose of 0.0005 mg. This phenomenon was termed by them "immunological paralysis". They also showed that this paralysis was coincident with hypersusceptibility to infection, since a large dose of the polysaccharide antigen, itself relatively non-toxic, decreased the resistance of mice to pneumococcus infection. In FELTON's work the paralyzing dose preceded the infecting dose. In the work reported here it is noteworthy that this order of inoculations left the animal apparently unaffected in its resistance to *Candida* infection, whereas the inoculation of yeast cells concomitantly with, 24, 48, or 72 hours after the infecting dose resulted in enhanced dissemination of the infection in these animals (see table 7). A prime point of similarity between this work and that of the FELTON group is that the same results could be produced by the antigen (in this case the yeast cell inoculum) either in a single dose or in smaller daily doses the sum of which equaled the single dose.

The phenomenon can scarcely be one of ordinary anaphylaxis, since none of the uninfected mice died or showed any other symptoms of shock, though they received the same series of yeast cell inoculations as the infected group.

The phenomenon, however, has some of the characteristics of a hypersensitivity phenomenon. The fact that cross-agglutinations between *C. albicans* and *S. cerevisiae* have been demonstrated (BENHAM, 1935) is in accord with such a hypothesis. In this connection the work of NAKAYAMA (1906) may be pertinent to this discussion.

NAKAYAMA found that guinea pigs receiving a second intraperitoneal injection of *Nocardia asteroides* (*Actinomyces asteroides*) one week after the primary injection died within 24 hours. This reaction was ascribed to sensitization and appears to be similar to anaphylatic shock. Both injections of *Nocardia* had to be made intraperitoneally for death to result. When tubercle bacilli were used for the primary injection and *N. asteroides* for the second the animals died within 24 hours. Sensitization did not develop in NAKAYAMA's animals until a week after the first inoculation and persisted for 3 to 4 weeks, after which the animals became refractory again. As a result of this study NAKAYAMA suggested the possibility that infection (at least in actinomycosis) results, not from a single exposure to a pathogen, but from repeated exposures leading to sensitization.

This study was later taken up in more detail by MATHIESON et al. (1935), whose observations essentially confirmed the findings of NAKAYAMA. MATHIESON and his workers, using *Actinomyces asteroides*, showed that both experimental infection and vaccination tend to produce an "allergic sensitization" in rabbits and guinea pigs. For example, when a guinea pig was inoculated intraperitoneally with 2 ml of a pure culture suspension of *A. bovis*, and 9 days later with 2 ml of washed crushed granules of *A. bovis* of the same strain as previously used, the animale died 8 days after the second inoculation. Autopsy revealed a generalized fibropurulent peritonitis. It may be significant that the animals in NAKAYAMA's work died soon after the second injection was given, but that in MATHIESON's work there was a delay of more than a week before the animals died. The findings as to the time of death of the *Candida*-infected animals that are reported here are more in accord with the data of the MATHIESON group. Mice infected with *C. albicans* and injected intraperitoneally with a single large dose of washed, living yeast cells showed a marked dissemination of systemic moniliasis as compared to infected controls, but only approximately 50 % of the animals given the yeast cell inoculation were dead after 20 days and very few died approximately 24 hours after the yeast cell injection (see table 7).

A practical application arising from this work would appear to be the ease with which a systemic *Candida* infection can be readily induced in mice in a relatively short period of time. This may be a helpful tool in evaluating various therapeutic agents, especially if this enhancement of infection can be shown to occur with others of the yeastlike pathogenic fungi.

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Candida albicans Infections in Actively and Passively Immunized Animals[1]

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Candida albicans (Robin) Berkhout, 1923, a yeast-like organism, is the causative agent of human moniliasis, a disease syndrome embracing a number of manifestations which have often been considered unrelated. The fungus may produce lesions in the mouth, vagina, skin, nails, bronchi, or lungs and more rarely, septicemia, endocarditis and meningitis. In the domestic rabbit, the experimental animal chosen for this work, the primary organ attacked is the kidney. Lesions are also produced in the liver, spleen, heart, lungs and intestines.

Grossly, the kidney and to a lesser extent the brain, heart and liver of rabbits infected with *C. albicans* show numerous, small, white abscesses scattered over the surface. Perhaps the work of Mackinnon (11) gives the best description of the disease in experimental animals. Intravenous inoculations in large doses produces death in from five days to a week. Autopsy shows small abscesses in all the visceral organs, but especially in the cortex of the kidney. In smaller doses many animals survive 8—10 days and sometimes longer. After 10—15 days, autopsies show exclusively renal lesions affecting the collecting tubules. In general these lesions are triangular in shape and marked out by abscesses 1—2 mm in diameter. These lesions leave traces of fibrosis. Cecil (3) describes the pathology of human monilial lesions as focal areas of necrosis in which are found budding cells with or without mycelium. He says that giant cells may or may not be present. The cellular reaction is one of polymorphonuclear leukocytes, monocytes and lymphocytes.

Jacobson and co-workers (9) in their text give a rather thorough description of the lesions in animals.

While much has been published on moniliasis, there is a dearth of material concerning actual immunity and the disease. Connor (5) working with organisms isolated from a case of osteomyelitis, noted that in a vaccinated animal there were no frank abscesses; rather he found small, acute tubercle-like lesions with *Candida* as the ,,common invader''. Small giant cells were numerous in the lymph nodes and kidney lesions. Whether this difference in the type of reaction was caused by the vaccine or not, the author was not certain; but he expressed the thought that a slight relative immunity might be shown in this way.

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[2] A part of this investigation was used in partial fulfillment of the requirements for the degree of Master of Science, June, 1950.

CATELLANI (2) very briefly suggests that *Monilia* (*Candida*) vaccines are useful in only a few cases but does not say that there is any particular type of moniliasis which responds to the vaccine. According to JACOBSON (9), autogenous vaccines made from organisms have been employed with success by GROSSI and BALOG in the treatment of a series of cases of bronchopulmonary moniliasis. In 1924 MARTIN (12) reported a case of pulmonary moniliasis which was treated successfully with immune serum. In this case there was a negative skin test, a negative agglutination, but a positive FOSHAY test.

HOFF (8) in his studies on *Cryptocossus hominis* (*C. neoformans*) reported immunizing mice by the injection of heat killed organisms. While both sets of mice died in approximately the same period of time, histological examination of lesions in the earlier stages showed immunity epxressed as a hastening of cellular proliferation with subsequent scar tissue reaction and the disappearance of the organisms. ZIMMERMAN (15) in reporting a case of meningitis due to *Candida albicans* suggested that some immunity may have been developed which played a part in the course of the disease.

The purpose of this work was to study the effect of antiserum on the course of the disease in experimentally infected rabbits. Since there is the possibility of sensitizing an animal through the use of vaccines, a method of passive immunization was tried. Special attention was given to histological differences between infected normal animals and infected immunized animals.

Passively and actively immunized animals as well as normal controls were given minimum lethal doses of a suspension of *C. albicans* and comparisons were made between tissue sections of the various groups. Comparisons were also made between groups of animals subjected to different experimental procedures, and consideration was also given to survival time by regrouping the animals and comparing those that survived for the same lengths of time regardless of the experimental procedure so as to indicate whether any differences observed were due to the experimental techniques or whether they might be due to a greater survival time of some of the rabbits.

Experimental Methods

Three different strains of *Candida albicas* were isolated and identified by following the procedures given by CONANT ET AL (4). After testing for virulence by intravenous injection into the marginal ear vein of a rabbit, a strain isolated from a female patient with chronic oral thrush was chosen for further work. Although this strain was highly pathogenic for rabbits and gave typical kidney lesions, it showed little or no pathogenicity for mice, which is in agreement with other reports on animal pathogenicity of *C. albicans*.

Vaccines prepared from a young culture 24 hours old and an older one of four days were suspended in sterile physiological saline solution (0.85 % NaCl). These suspensions were then heated to 60° C. for thirty minutes, cooled and 1 ml. of 10 % formalin was added to each 100 ml. of suspensions as a preservative. The suspensions were then tested for sterility by streaking modified Sabouraud agar plates. At first a suspension of a density equal to a MCFARLAND number three was used but this was later increased to about a MCFARLAND number six. The dosage varied from 0.5 ml. to 2.5 ml. and was injected into the marginal ear veins of the rabbits. MCKINNEY (13) states that it is more difficult to immunize rabbits against the yeast-like organisms than against bacteria, and the authors of this paper concur with this, hence the increase in density of the suspension and volume injected. DRAKE (6) found hist to be even more striking in work with *Cryptococcus neoformans* since with this organism, large doses of dense suspensions are necessary to assure antibody response in rabbits. The use of small numbers of organisms for immunization has probably been responsible for the erroneous reports that this latter organism is not antigenic.

When the titer was to be tested, the animal was either bled from the ear or anesthetized and bled from the heart. When the titer had reached 1 : 2560, the rabbits were bled under sterile conditions and the serum stored in the refrigerator until needed. No preservative was added.

In testing for agglutinins a method suggested by Norris (14) was used at first but this was later replaced by a method described by Drake (6) in which the test is read macroscopically on Kline agglutinations slides within 15 minutes after mixing antigen and antiserum. If spontaneous agglutination occurred with the control, the test was repeated.

At death the animals were autopsied and the various organs examined for lesions. Modified Sabouraud agar plates were streaked from the organs having lesions, and parts of the organs were fixed in 10 % formalin for sectioning later. After washing, the tissues were dehydrated either with a dioxane or acetone method and them embedded. Sections were cut from 7 to 10 microns in thickness.

Several methods of staining were tried. One of each of the sections was always stained with a standard hematoxylin-eosin method. In addition a method was sought which would differentially stain the fungus in the tissue. Fuentes' (7) technic, which he considered superior to the Gram method, was tried but without success because efforts to dissolve Sudan IV (scarlet red) in pure lactic acid were unsuccessful. Three different modifications of the Gram stain were tried and used to some extent to supplement the hematoxylin-eosin staining. These were the amended Gram-Weigert, Mallory's variation (10) and MacCallum's modification of Goodpasture's method (4).

Experimental Results

The MLD for these experiments was a one ml. suspension of sufficient concentration as to bring death on the seventh day. This was established by preliminary tests using normal rabbits.

The first group of experiments was divided into five parts using a total of 25 rabbits. A series of 10 rabbits was used, each one being given one MLD. In the second series of five animals each was given one ml. of antiserum (titer 1 : 2560) and immediately followed by the injection of one MLD. In the third series of five rabbits, each was given three mls. of antiserum and one MLD immediately afterwards. In a fourth series, which was started some time later, three rabbits were given one MLD and two of these three were given regular intravenous injections of antiserum. In the fifth series the two rabbits which had been used for the production of the antiserum were given one MLD each. Later other experiments were carried out to try to answer some of the questions which arose from the findings of the first group of experiments. These will be briefly outlined in a later section.

In group one, eight of the ten rabbits which received one MLD died within a week. Seven of these showed the pathology as described by Jacobson (9). The eight animal (rabbit 12) had a very small, well circumscribed mass in the lung composed of several dozen macrophages enclosing a single blastospore. Small abscesses were seen in the kidney with mycelium scanty to abundant in amount. The macrophages were the predominating cell type. Some of the abscesses contained packed masses of organisms, chiefly mycelium. These lesions were fairly well circumscribed, but not walled off from the surrounding tissue of which there was invasion at times. In three other rabbits of this group the organisms were packed and more abundant in the periphery of the lesion. Rabbit 12 showed masses of organisms with pink material in between, holding together the mass from which organisms were still growing and extending outwards. Such masses gave a definite impression of an actinomycetoid ("sulfur") granule type of growth, although the radiating arrangement was not as yet well developed. Many lesions were present in the brain with macrophages predominating in some and polymorphonuclear leucocytes in others; mycelium was much more abundant than blastospores. Numerous diffuse lesions showed invasion into the surrounding tissue. Phagocytosis was very rarely seen. The predominating form of the organism in the heart lesions was blastospores in rather large numbers. Although many of the organisms were phacocytized, this process was not controlling the invader.

All of the eight rabbits showed numcerous liver abscesses but the organisms were sparse in number and present mostly as blastospores. The spleens showed a few scattered organisms which had recently invaded or metastasized without noticeable cell response.

In group two, three of the five rabbits, which were given one MLD and one ml. of antiserum, died in from five to six days. The kidney abscesses showed an unusual amount of necrotic phagocytes and contained blastospores which were often very large with thick walls. In some areas a walled-off central portion of the abscess contained polymorphonuclear leucocytes as well as macrophages and there was in the central portion of some an exceedingly well developed and typical actinomycetoid granule which stained very strongly with eosin. Less well developed granules were seen in some of the other abscesses. It should be noted that the actinomycetoid structure had developed before epithelioid cell development. Many lesions had, some place within the central portion, a mass of tightly entwined, twisted mycelium appearing to be the early stage in the formation of "sulfur granules". There was some connective tissue proliferation but it was still minimal. In the brain there were multiple small and large abscesses with mycelium predominating as short strands, often rather extensively branched and there was some invasion into the surrounding tissue. The predominate form of the organism in the heart was thick-walled blastospores; however, a few lesions showed a very early mycelial stage or else an abortive attempt to form mycelium. The blastospores, some of which appeared to have thick, striated walls or else were very finely lobulated, seemed unusually large and some seemed to be phagocytized.

In group three, four of the five rabbits which were given one MLD and three mls. of antiserum died in from five to six days. The kidney of rabbit 18 contained thick walled blastospores in the lesions. The lesions of rabbit 20 suggested a very early stage of granulomatous tissue, often with necrotic centers, and the macrophages, particularly at the periphery, suggested epithelioid cells. The heart lesions of rabbit 20 showed blastospores, scanty in number, with unusually thick walls; some appeared to be phagocytized. The other two rabbits showed the same general findings as described by JACOBSON.

The rabbits in group four (rabbits 26 and 27) were given one MLD and daily injections of antiserum until they died, which was within a week. Rabbit 27 died on the fiftieth day. Many of the larger abscesses in the kidney cortex of this animal showed little evidence of degeneration and often contained bright eosinophilic amorphous masses in which were embedded blastospores and short strands of mycelium. One abscess contained a packed mass of mycelium and blastospores but these were not sufficiently radiate for a sulfur granule. Rabbit 26, which died in seven days, had multiple abscesses throughout the kidney cortex consisting of a rather dense collection of macrophages with some connective tissue proliferation; the lesions appeared to be developing a tubercle-like structure, although no giant or epithelioid cells were seen. Central masses of some of the lesions were composed of strands of mycelium. Although there was more evidence of repair than usual in the heart muscle, a general impression was that the lesions, which contained a moderate number of elongated blastospores and short strands of mycelium, were larger in size and fewer in number than ordinarily observed. Thick walled blastospores were seen in some of the liver lesions of rabbit 26.

In group five, two rabbits, rabbits 1 and 2, were given injections of antigen beginning December 2, 1949, and continued until August 26, 1950, at which time rabbit A had an agglutin titer of 1 : 2560 and rabbit 2, a titer of 1 : 1280. On September 2, 1950, they were given one MLD. They died in less than 48 hours. Morphologically the organisms, frequently seen invading the kidney tissue, consisted chiefly of unusually short and multibranched strands of mycelium. In some of the heart lesions short strands of mycelium were invading the surrounding tissue. Blastospores, when seen, seemed unusually large and somewhat thick-walled. In some of the lesions of the rabbit which lived a few hours longer, rabbit 1, a number of the organisms appeared to be growing in very tight masses. This rabbit also had some young lesions with elongated blastospores and a portion of the granular and vacuolated mycelium was in many cases pseudomycelium. In the liver lesions were seen thick-walled blastospores and short strands of mycelium which were somewhat distorted and twisted and often varying in diameter. New lesions appeared to be arising out in the cord cells rather than in regions of fibrosis (these fibrotic areas were probably the result of the large number of injections of antigen.)

Group six. Since these two rabbits, 23 and 25, which received one MLD and one ml. of antiserum on July 27, 1950, had not died, they were killed. In the tissues there were areas of fibrosis which contained scattered macrophages and appeared to be healed and entirely inactive lesions. In other areas fibroblasts and macrophages were the predominating cell type, fibrosis was distinct. There were no organisms seen.

In group seven, rabbit 10 was killed on September 3, 1950, since it had not died after receiving one MLD on Juny 27, 1950. Any lesions which were present appeared completely inactive.

In group eight, rabbit 13 which was given one MLD on July 27, 1950, and another on September 2, 1950, died on September 6, 1950. In the kidney were only a moderate number of dense abscesses present in the cortex with macrophages and degenerating polymorphonuclear leucocytes and rather eosinophilic granules. A moderate number of organisms were present in the abscesses either as blastospores, some of which appeared elongated, or as short strands of mycelium often appearing somewhat degenerated with a great variation in size and thickness of strands and vacuolization. The organisms were present in the brain lesions in only moderate numbers as blastospores and short strands of very granular mycelium. Fairly numerous abscesses were seen in the heart muscle and contained macrophages and abundant polymorphonuclear leucocytes, which sometimes appeared to be degenerating. The organisms were present as short strands of mycelium and thick-walled blastospores, some quite large and some phagocytized. The liver lesions appeared rather young and active. The only type of organisms seen were blastospores, some of which appeared either very large or very small; these were frequently distorted and often appeared to be somewhat degenerating as evidenced by the marked variation in size.

Rabbit 19 (Group nine) was kiled on August 6, since it had not died after receiving one MLD plus three mls. of antiserum on July 27. There were only minimum inactive lesions with fibroblasts, connective tissue fibers, usually some macrophages. The lesions appeared to be almost healed.

In group ten, for comparison rabbits 31 and 32 were each given one MLD. Rabbit 32 was killed after 24 hours and rabbit 31 was killed after 48 yours. The kidney of rabbit 32 contained small cortical abscesses which possessed occasional blastospores and moderate amounts of both pseudomycelium and true mycelium. There was some invasion of the surrounding tissue. The cortical abscesses in rabbit 31 contained macrophages and degenerating polymorphonuclear leucocytes with an unusual abundance of organisms present as mycelium and blastospores which in one case formed a rather tightly packed mass. Similar small lesions were seen in the medulla and in some cases organisms could be seen invading the surrounding tissue.

Rabbit 30 and rabbit 29, group eleven, were each given one MLD and five mls. of antiserum. Rabbit 30 was killed after 24 hours and rabbit 29 after 48 hours.. Rabbit 30 had diffuse small long abscesses with necrotic centers, degenerating polymorphonuclear leucocytes and a very few macrophages. Even in this early stage the macrophages tended to pack very tightly together. While some of the organisms appeared to be degenerating, others had exceptionally heavy walls as if surrounded by a capsule. An organism appearing in a lung lesion definitely showed a radiating edge with prickles around the blastospores. Rabbit 29 had many abscesses, small, medium and large, in the kidney cortex with macrophages as the predominating cell type. It was very striking that almost every lesion contained a solid mass of organisms often embedded in an eosinophilic matrix. Of these, both blastospores and mycelium intermixed, none showed radiations. Blastospores and pseudomycelium predominated in occasional small abscesses of the medulla, but in others the greatest bulk was in packed organisms. The liver of rabbit 29 showed a large number of small abscesses with the organisms chiefly present as thick walled blastospores and very short strands of mycelium, which did not appear to be particularly abundant although found in all of the lesions. The heart contained numerous small, medium and large sized abscesses with the organisms present as branched mycelium and blastospores.

Discussion of Results

Using the survival time of the various animals as a basis for grouping, we may compare the findings as follows:

One day: If we take rabbit 32 as an example of the usual pathological changes caused by one MLD, then the variations in the rabbit receiving the antiserum are probably brought about by the antiserum. The short branched mycelium in the kidney of rabbit 30 would not be expected as normal finding and neither would the dense, early packing of macrophages in the spleen and lung. The degeneration and eosinophilic nature of the organisms in the lung and liver, the short, branched mycelium and thick walled blastospores in the lung, one of which definitely showed a radiate edge, were all confined to the serum treated animals and were not found in the control.

Two days: While the kidney of the non-immunized rabbit had one rather tightly packed mass of organisms, it is of interest to note that almost every lesion in the immunized rabbit contained a solid pack of organisms often embedded in an eosinophilic matrix. While orgnaisms were present in both, there was a difference in their morphology, rabbit 31 having blastospores and short mycelium, and rabbit 29 (with antiserum) having branching mycelium in some cases, and thick-walled blastospores in others. There appeared a tendency towards smaller lesions in the nonimmunized rabbit.

Five days: Here a non-immunized rabbit showed some of the characteristics one would expect in an antiserum treated rabbit. A few blastospores with thick walls in the lung and the packed organisms giving a "sulfur granule" type of lesion in the kidney were seen. It may be that the animal had some naturally occurring antibodies, Drake (6), or that a titer of sufficient potency had been built up to cause these changes. From the findings in the passively immunized rabbits it may be surmised that the antiserum brought about the changes in the morphology of the organism-thick-walled, unusually large blastospores, some of which had striated edges or were very finely lobulated. It was in this group that the actionomycetoid granules were found. It is speculative whether the larger lesions were from the effect of the antiserum or not.

Six days: Again the immunized animals showed the large thick-walled blastospores and the tendency for the organisms to form packed masses as in the beginning of a "sulfur granule". Another difference to be noted is the necrosis occurring in the centers of many of the lesions, a change not noted in the non-immunized rabbits. At this stage, the lesions in the kidney of the rabbits which received three mls. of antiserum closely resembled the lesions described by JACOBSON (9) in that they were of the granulomatous type.

Seven days: In seven days there were solidly packed massed organisms in the kidney of a non-immunized rabbit. One could ask the question, ,,Was this the result of a normal progress or were there antibodies present in small amounts which may have had an effect"? The typical clubshaped projections, radially arranged and the tubercle-like structures were found only in treated rabbits.

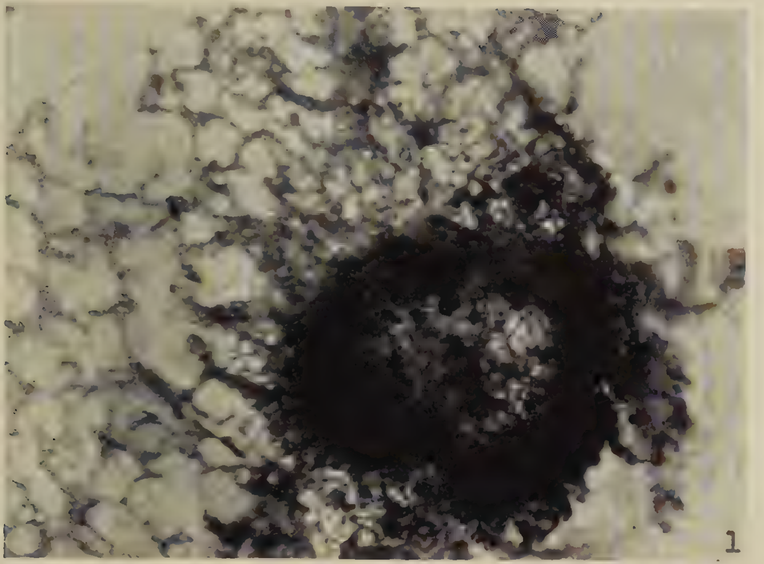
In the animal which lived a month or longer some of the lesions showed the same picture as was found in the immunized rabbits of a week or less survival. Rabbit 13 which lived 41 days and had been given two MLD's showed a positive titer just before death as did four other rabbits which had been given one MLD and lived for a period of time. The two actively immunized animals were seemingly not protected or else their early death might have been due to a tuberculin type of shock (1).

Summary and Conclusions

1. The literature on the pathology and immunology of *Candida albicans* is briefly reviewed.
2. Experimental methods and techniques used in this study are presented.
3. Experimental observations described here show that:
   a. Many of the lesions found differed from those described by CECIL and JACOBSON.
   b. The immunization did not seem to preserve the life of the animal nor did it extend the survival time over that of the controles, the general impression being that it was not only ineffective but possibly deleterious.
   c. The antiserum seemed to have some effect on the morphology of the organisms shown by the multibranched mycelium, the thick-walled, distorted blastospores, the heavy packing of the organisms in some cases and the presence of typical "sulfur granules", the actinomycetoid form of the organisms, in a number of animals.
   d. The antiserum seemed to have little or not effect on the tissue reactions.

Sommaire et Conclusions

1. La littérature concernant la pathologie et l'immunologie de *Candida albicans* est brièvement passée en revue.
2. Les méthodes d'experimentation et les techniques employées dans cette étude sont presentées.
3. Les observations faites pendant les experiences montrent que:
   a. Beaucoup des lésions trouvées differaient de celles décrites par CECIL et JACOBSON.
   b. L'immunisation n'a pas semblé préserver la vie de l'animal ni prolonger la période de survie au dela de celle des animaux de contrôle. L'impression générale est que l'immunisation fut, non seulement inefficace, mais peut-être nuisible.
   c. L'antisérum semble avoir eu, sur la morphologie des organismes, quelque effet démontré par le mycélium multibranche, les blastospores tordus



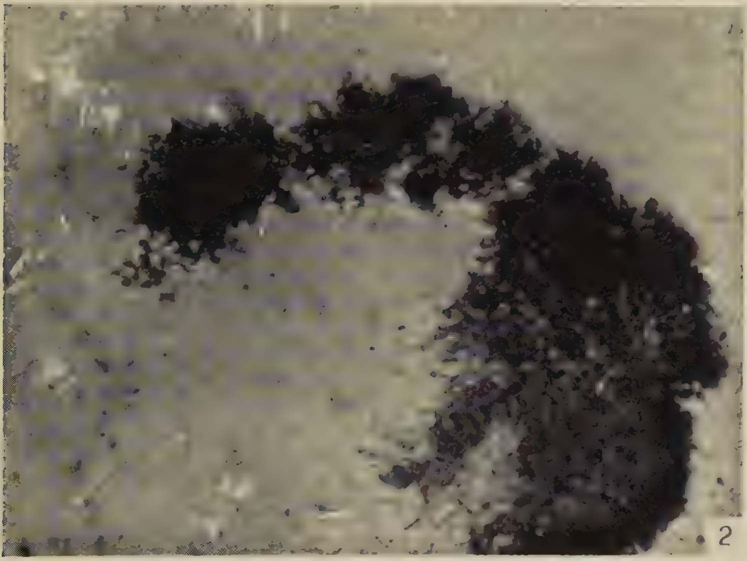
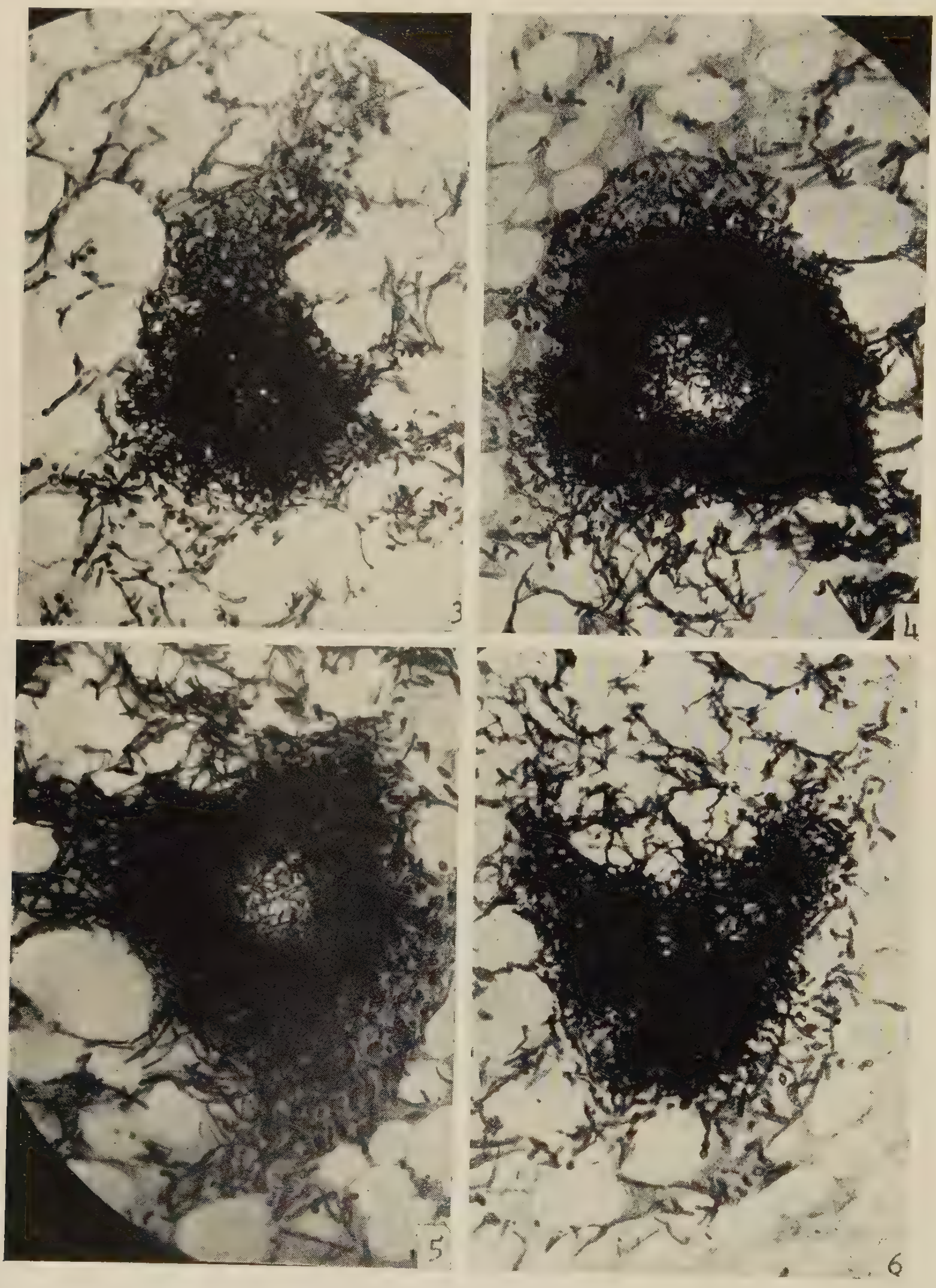
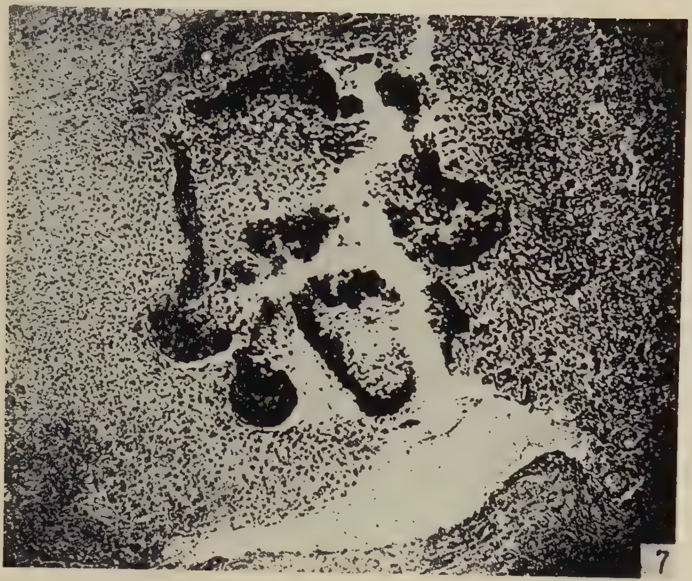


Fig. 1. Cross-section of a lesion produced in the mesenteric adipose tissue of a mouse injected with *Candida albicans* in saline suspension. Paraffin preparation, picro-formol-acetic fixation, McManus-Hotchkiss stain. × 440.

Fig. 2. Similar structure from the spleen of a rat inoculated intraperitoneally with 1 cc of a 1 : 100 suspension of *Candida albicans* in 5 % mucin. Paraffin preparation, picro-formol-acetic fixation, McManus-Hotchkiss stain. × 440.



Figs. 3—6. Cross-sections of one of these lesions (over-all dimensions $75\mu \times 50\mu$) in the adipose tissue of a mouse at four different levels. Fixation and staining as in figs. 1 and 2. × 440.



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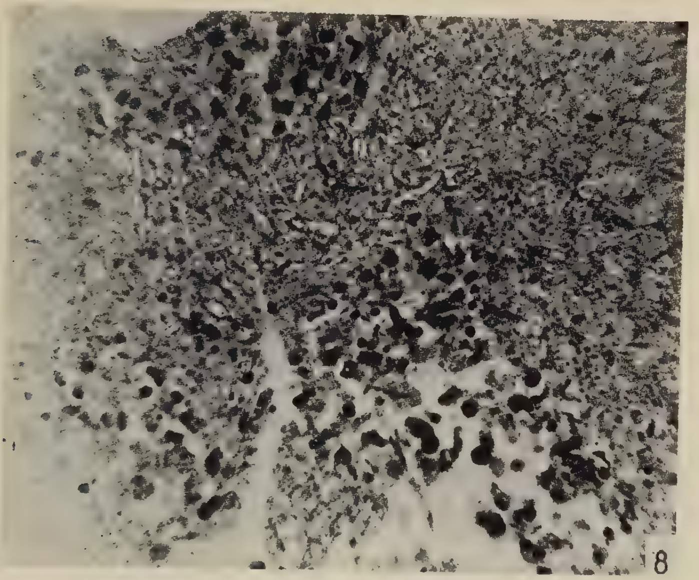

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Fig. 7. Granuloma production by *Torula jeanselmeini* after Langeron.

Fig. 8. Leucocytic infiltration at the site of production of the lesion pictured in figs. 3—6 Paraffin preparation, picro-formol-acetic fixation, hematoxylin and eosin stain. × 900.

aux parois épaisses, dans certains cas un conglomérat très dense des organismes et la présence de typiques "sulfur granules", la forme actoinomycetoide des organismes chez un certain nombre d'animaux.

d. L'antisérum semble avoir eu peu ou pas d'effet sur la réaction tissulaire.

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Granule Formation in Animal Tissues by *Candida albicans*[1]

by

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(Plates XLV-XLVII)

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The occurrence of granules in the tissue of the host is relatively frequent in fungus infections. Although the cause of the disease was not recognized, the first case of a foot mycetoma was noted by COLEBROOK (1) in 1876 in Madura, India — hence called by him "Madura foot." When the real nature of the disease was ascertained, and more cases were known, it was found that this appellation was misleading. The disease, although not of very frequent occurrence, is widespread, and is not limited to the foot, but can occur in many other sites, such as the arms, the cervicofacial region, the thorax and the abdomen. All of these growths are called mycetomas. Although *Nocardia*, the aerobic form of *Actinomyces*, is most frequently responsible for these pathological conditions, many other fungi have also been cultivated from mycetomas. In this country they are frequently caused by *Monosporium apiospermum*, which was shown by EMMONS (3) to be the imperfect stage of *Allescheria Boydii*.

Candida albicans is known to be a frequent cause of superficial skin infections. There are two cases reported by ROCKWOOD and GREENWOOD (6) and by MIALE (5) which indicated that the reaction of the tissue to *Candida albicans* may be that of chronic inflammation and these two cases are cited in this connection by CONANT (2).

Incidental to our own study of chronic changes induced by *Candida albicans* in experimental animals, granule formation in internal organs has been observed. However, such structures in the course of infection with *Candida albicans* are relatively infrequent. Among several hundred animals studied we have seen them only twice. Time seems to be one of the most important factors in their development. In both cases referred to, the duration of the infection was three to four weeks. The largest granules were observed in a mouse injected with *Candida albicans* in saline suspension, which died three weeks later.

Examples of these configurations appear in the accompanying photographs. They are shaped like a hollow cylinder closed at the ends, and are consequently ringshaped in cross section. Such growths could sometimes be seen in mice of short survival, but these were not characterized by the internal cavity which appears as a "ring" in cross section. Figure 1 represents a granule in a lesion caused by *Candida albicans* in mesenteric adipose tissue; figure 2 shows a similar structure in the spleen of a rat, inoculated intraperitoneally

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with 1 cc. of a 1 : 100 suspension of *C. albicans* in 5 % mucin, which showed slight symptoms of disease and was sacrificed 32 days after inoculation. Serial sections of the adipose tissue shows that there is considerable variation in size of the granules. The smallest one measures 50 μ across the ends and 75 μ in mid-diameter. Its length is 180 μ. The diameter of the middle-sized granule is 90 μ at the ends and 135 μ in the middle, and its length is 190 μ. Furthermore, we found others which are much larger, sometimes reaching a diameter of 210 μ at the widest part and more than 300 μ in length. Cross sections of one of these at four different levels appear in figures 3—6.

The morphological appearance of these granules is similar to that described in the mycetomas mentioned above. LANGERON observed (4) lesions caused by *Actinomyces madurae* and *Torula jeanselmeini* (nomenclature of M. LANGERON). He also observed that component parts of the lesions sometimes overlie one another, and this morphological appearance led him to call them "*granules vermicularis.*" Leucocytic infiltration can be seen in hematoxylin and eosin stained preparations (figures 7 and 8).

Perhaps it would not be out of place to note here that the tendency to grow in a special pattern is an attribute of fungi under many other circumstances. The best example in human pathology is "ringworm," the circular skin lesion produced by a number of different dermatophytes. Whereas the extent of development in the third dimension is definitely limited in a thin tissue such as skin, internal organs offer the opportunity for more extensive three-dimensional growth and the above described changes in deeper tissues of experimental animals are possibly but another expression of the same growth pattern.

We might mention here briefly other cases where fungi on quite different substrates also have a tendency to "ring" formation. For example, *Streptomyces* sometimes produces rings on artificial media. An interesting parallel is the production by mushrooms in the open fields of "fairy rings", so named by the non-scientists who first observed this interesting phenomenon.

Summary

1. Granule formation by *Candida albicans* in the internal organs of experimental animals is described. This appears to be the first time such an observation has been made.

2. Ring formation by fungi under different conditions is cited, and although different explanations have been proposed by different authors, the supposition is made that a common growth pattern is involved.

Acknowledgments

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The Effect of a Concentrate of Nocardin on the Growth of Streptomycin Sensitive and Resistant Strains of *Mycobacteria Tuberculosis*

by

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(2.5.52)

In 1947 a new tuberculostatic antibiotic, Nocardin, was reported from the Actinomycete, *Nocardia coeliaca* (Gray and Thornton) Emmons. This was found to be active against human tubercle bacilli *in vitro*; to inhibit tubercle development on the chorio-allantoic membrane of the chick and to partially inhibit the loss of weight in tuberculous guinea pigs (1,2). A crude product extracted from the mycelium was later found to inhibit the development of tubercles in the lungs of tuberculous mice (3). These early findings on the tuberculostatic activity of Nocardin were later verified and the bacteriostatic action *in vitro* extended by Levaditi (4) and Levaditi and Henry-Eveno (5).

The present report is an account of the activity *in vitro* of a more highly purified form of Nocardin. While as yet not in crystalline form, nevertheless, this fraction is water soluble and stable in solution over a two year period. Its activity *in vitro* against four human strains of tubercle bacilli and their streptomycin resistant biotypes is reported herewith.

Materials and Methods

The determination of the tuberculostatic activity of the original culture filtrate from the Nocardia cultures as well as the antibiotic fractions at various stages of purification was made with the standard strain H37 Rv. After two years of experimentation in the fractionation and purification of the antibiotic, a stable, water soluble fraction was obtained. This fraction was then also tested against a streptomycin resistant biotype H37 Rv. The resistance of this strain was induced by subjecting the strain *in vitro* to increasing concentrations of streptomycin until the degree of resistance was a thousand fold. Three other pairs of strains of tubercle bacilli, designated as "F", "H", "A" strains were similarly used. These were isolated from patients before treatment while those resistant to streptomycin, isolated after treatment was begun were designated as "FR", "HR" and "AR". All strains were received from the Trudeau Laboratories. The control strains were sensitive from 0.1 to 0.2 gamma of streptomycin per milliliter of culture medium, while the resistant strains tolerated a thousand times as much streptomycin.

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These strains have been maintained for the past two years in Kirchner's medium containing 2 % sucrose and a trace of iron. The bacilli were harvested from the pellicle of stock cultures three to four weeks old. Homogeneous suspensions were prepared for inoculation of the experimental cultures by means of a White bacterial grinder. The concentration was calculated by moist weight of the pellicle, each ml. containing 1.5 mg. of bacilli. Since the total volume of each tube was 10 ml., this gave a final dilution of 0.15 mg. per ml. for starting concentration of the bacilli for all experiments. With this concentration it was possible to obtain measurable differences in turbidity on the fourth day after inoculation. The mean daily readings were calculated from triplicate cultures containing the antibiotic in serial dilution and the percent growth of the treated bacilli calculated on the basis of the growth in tubes receiving no Nocardin. Turbidimetric determinations were made using the Coleman photonephelometer at a wave length of 590 mμ. The mean percent growth for all strains studied in serial dilution of the antibiotic was based on four to seven experiments in which each concentration was run in triplicate.

Culture medium for the in vitro tests to determine tuberculostatic activity.

For the study of growth under control and experimental conditions the strains of tubercle bacilli were grown in submerged culture in medium prepared as follows:

Part I	Na_2HPO_4 (anhydrous)	3.0 gms.
	$K\,H_2PO_4$	4.0
	Sodium citrate.	2.5
	$Mg\,SO_4.7H_2O$	0.6
	Asparagin	5.0
	Glycerin.	10.0
	Sucrose	20.0
	Water	1000.0

The pH was brought to 7.0 by adding 1.5 ml. of 40 % NaOH. To each 1000 ml. of the above solution 2.0 ml. of a 10 % solution of Tween 80 were added. This part of the medium was sterilized by autoclaving at 15 lbs. pressure for 30 minutes.

Part II

A second part of the culture medium consisted of a solution of vitamins, prepared as follows:

Vitamin	*Mg. %*	*Vol. ml.*
Niacin	20.0	50.0
Para-aminobenzoic acid	100.0	10.0
Biotin	2.5	40.0
Pyridoxine	100.0	10.0
Thiamin	100.0	10.0
Calcium pantothenate	100.0	10.0
Folic acid	40.0	20.0
Riboflavin	5.0	10.0
		160.0 ml.

Each of the vitamin solution was passed through Seitz filters and combined immediately before using. Ten ml. of the vitamin mixture was added to each 70 ml. of sterile base medium containing Tween 80.

Part III

A third part of the medium consists of serum albumin (Bovine fraction V — Armour). This was prepared in 2 % solution and heated to 56° C. for 10 minutes, cooled and sterilized by filtration. Twenty milliliters of this were added to 80 ml. of the base culture medium containing vitamins. No further adjustment in pH was necessary.

The above formula has been continually used in this laboratory over the past four years for the study of many strains in deep culture. Over the standard eight day culture period, continually used in all experiments, all cultures studied maintained a homogeneous milky turbidity without macroscopic clumping.

Culture medium for the growth of Nocardia coeliaca

The mold was cultured over a period of 6 weeks at 37° C. in large flat bottom flasks, 1000 ml. to a flask. The following formula was used in preparing the medium.

Dibasic sodium phosphate (anhydrous)	3.00 gms.
Monopotassium phosphate	4.00
Magnesium sulphate	0.60
Sodium citrate	2.50
Asparagin	5.00
Glycerin	20.00
$FeSO_4.7H_2O$	0.01
Sucrose	20.00
40 % NaOH to give a pH of 7.2	2.00 ml.
Water (triple distilled) to make	1000.00 ml.

The flasks were autoclaved at 15 lbs. pressure for 30 minutes. The cultures were started by planting with spores of Nocardia from a 4 to 7 day culture. The spores spread rapidly over the surface and the mycelium readily covered the surface in about ten days. The medium was sufficiently rich in nutrients to support sporulation which normally recurs approximately every fourteen days. With growth of the mycelium the clear culture medium becomes reddish brown, the maximum antibiotic activity being obtained with this medium in about 5 to 6 weeks. Cultures were set up 1000 ml. per flask, and 20 to 40 flasks per experiment. At the end of the culture period the cultures were pooled, the volumes measured, acidulated to pH 5.5 and autoclaved at 10 lbs. pressure for 20 minutes. The fluid was then filtered off the mycelium and the active substance washed from the mycelium with hot water (90° C). This latter was added to the filtrate and the whole evaporated to the total volume of the original filtrate. A portion of this was set aside, the pH adjusted to 7.0, the medium filtered through Seitz filters and tested *in vitro* for the tuberculostatic activity. The dry weight per ml of the filtrate was also obtained. The activity of this unpurified portion formed a standard of comparison for testing the fractions at various stages of purification. The present report presents the comparison of the activity of the unpurified culture filtrate with the activity of a stable, water soluble fraction of high degree of activity but not as yet of crystalline purity.

Preparation of the Nocardin Concentrate

The active substance was washed from pooled mycelia with hot water, the eluate added to the pooled filtrates and the active substance absorbed on charcoal (Darco G-60). The charcoal was then eluted with 50 % ethanol and the eluate concentrated. The concentrate was then freed of neutral 90 % ethanol insolubles and alkaline barium-ethanol insolubles. The active fraction after removal of barium, was then precipitated with phosphotungstic acid and the precipitate freed of phosphotungstic acid with barium hydroxide. This was then freed of barium with sulfuric acid at pH 6 (Fig. 1). The solution was freeze-dried and then dissolved in water at 100 mg. per ml. and the pH adjusted to ph 6.5—6.8.

This preparation was further diluted so that 1 ml. = 1 mg. and the whole sterilized by filtration through Seitz filters. Fractions of Nocardin prepared in this manner have been maintained in solution at 6° C. for over 3 years without loss of potency.

Figure 1
Method of fractionation.

Starting material
(Pooled filtrates and aqueous extracts of mycelia.)
|
Absorption on charcoal (Darco-G-60).
|
Elution with 50 % ethanol
|
Concentration by evaporation
|
Removal of neutral alcohol insolubles by adding 90 % ethanol
|
Precipitation of basic insolubles by barium hydroxide in ethanol
/ \
Soluble fraction Precipitate
|
Barium removed with 5 % sulphuric acid and evaporated free of alcohol
|
Precipitation of soluble fraction with phosphotungstic acid.
|
Precipitate decomposed with barium hydroxide
|
Barium removed with sulphuric acid at ph 6.0
|
Final solution freeze dried and weighed.

Results

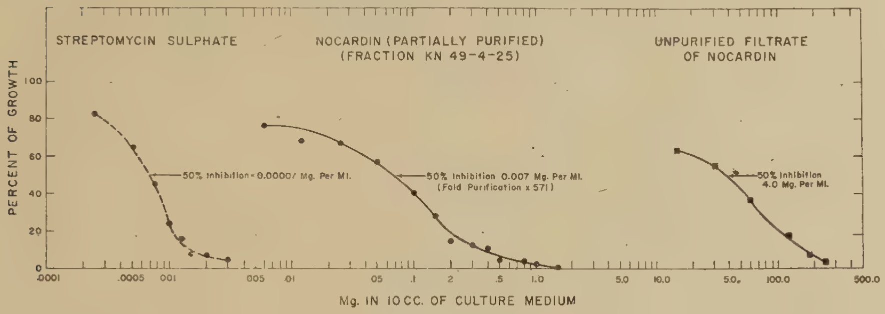
Relative Effectiveness of Partially Purified Nocardin and Streptomycin.

When the unpurified crude filtrate was tested against the tubercle bacilli (Strain H37Rv) in 10 ml. of culture medium and the percent growth calculated 8 days after inoculation, it was found that 4 mg. per ml. permitted 50 percent growth, while 10 mg. per ml. allowed only 20 percent growth. A comparison of these data with the curve obtained for the partially purified fraction shows that 7.0 gamma per ml. of nocardin gives 50 percent inhibition. This is a 571 fold purification (Chart 1).

A comparison was made under identical culture conditions of this latter preparation with streptomycin. Since streptomycin permitted 50 % growth at a concentration of .07 gamma per ml., this indicated that the crystalline streptomycin[1] was 100 fold more effective than the Nocardin preparation described above. Since 7 gamma per ml. of partially purified Nocardin gave 50 % inhibition, this degree of effectiveness of the antibiotic *in vitro* suggested that it be tried against other strains of tubercle bacilli, especially against streptomycin resistant strains.

CHART I

TUBERCULOSTATIC ACTION OF NOCARDIN FILTRATE, AND PARTIALLY PURIFIED NOCARDIN AS COMPARED WITH STREPTOMYCIN



While further improvements in culture media and other methods of purification might be undertaken to improve yield and potency of the product, it was thought that a study of the effectiveness of the partially purified antibiotic against different strains would yield further evidence of the activity of the antibiotic and justify the extension of the difficult task of purification. With this in mind a study of the action of Nocardin of paired streptomycin resistant and sensitive strains was undertaken.

Effectiveness of Nocardin Against Streptomycin Resistant and Control Strains.

(a) Difference in initial growth of untreated paired strains.

It has been previously shown statistically that streptomycin resistant biotype H37 RvR does not have the same growth pattern as the parent control strain, H37 Rv[2]. Since the growth as represented by the turbidity of the untreated strain at the termination of the experiment is the basis for calculation of the percent growth of the treated strain, a study of the difference in growth of the four pairs of control and resistant strains was made. The growth of strains "F", "H", and "A" and their resistant sister strains "FR", "HR" and "AR" was therefore studied *in vitro* under identical conditions in triplicate cultures in 4 to 6 experiments. Since it was not possible to measure precisely the turbidity during the lag period- the interval between inoculation and measurable growth- a comparison of the growth of the strains was based upon the mean turbidity readings of all tubes between the 4th and 8th day of all the experiments. Where no overlapping of growth curves occurred a difference in growth was evident. Although the differences may be slight, these differences are recognizable in growth curves of three of the streptomycin resistant strains. In strain H37 Rv, the sensitive strain consistently grew at a slightly higher rate than the streptomycin resistant strain. This was also true of the "A" and "AR" strains (Chart 2, Figs. 1 and 4). Similarly in the human strains A 27 and A 27R previously reported (6), it was found that the streptomycin resistant strain required a longer time to reach the same degree of turbidity than the sensitive strain. In the "H" and "HR" strains the sensitive strains had a lower growth rate than the resistant strain and in the "F" and "FR" strains no difference could be detected in their growth rates (Chart 2, Fig. 3). It would appear therefore that these differences in growth rate cannot be consistently associated with the phenomena of acquired resistance to streptomycin. Since all strains were systematically transferred on the same day and were therefore consistently of the same age when used, the differences shown probably represent slight genetic variations in growth pattern rather than variations due to experimental culture technique. While these differences are slight they are of importance when estimating the relative amount of an antibiotic which should be used to inhibit the growth of the strain.

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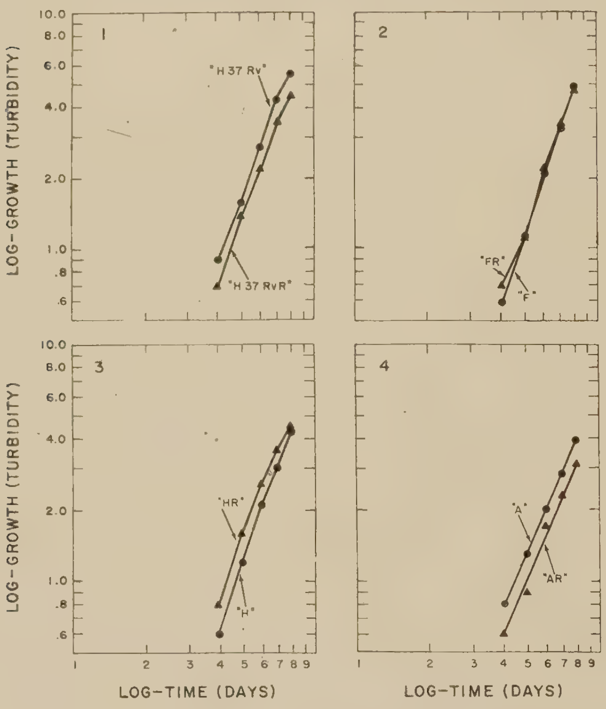
[1] Streptomycin sulphate (Pfizer).

[2] Unpublished data.

CHART 2

COMPARATIVE GROWTH IN VITRO OF 4 PAIRS OF STREPTOMYCIN RESISTANT AND CONTROL STRAINS OF MYCOBACTERIUM TUBERCULOSIS

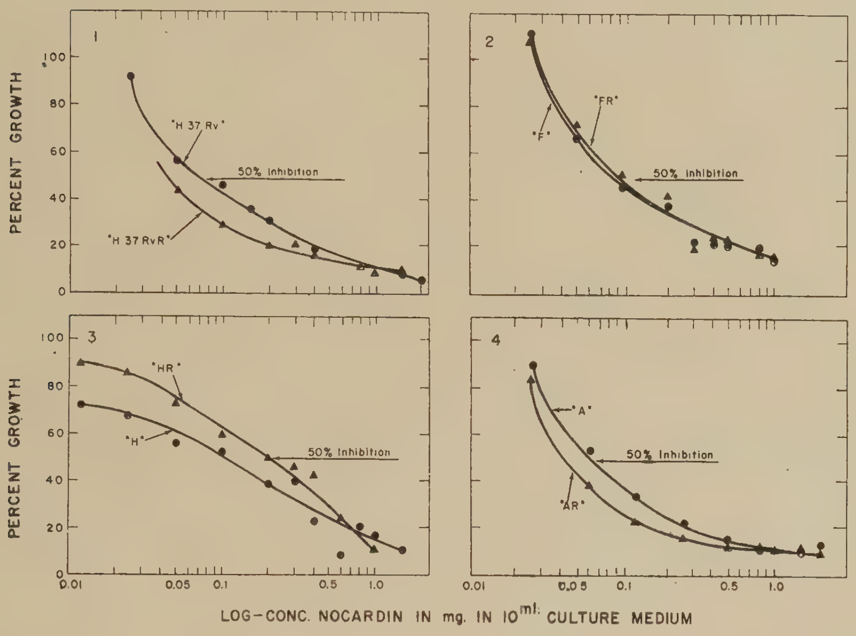


(b) The effectiveness of Nocardin in Inhibiting Resistant and Controls Strains.

In Chart 3 is shown the tuberculostatic action of partially purified Nocardin against four pairs of streptomycin resistant and control strains. Except for strain "H" and its resistant biotype "HR" which requires between 10 to 20 gamma per ml. to give 50 % inhibition, the three other control strains and their resistant biotypes show 50 percent inhibition in a concentration of 0.04 to 0.10 mg. in 10 ml. of culture medium or 4 to 10 gamma per ml. These curves (Chart 3) indicate that Nocardin is equally effective in inhibiting the streptomycin resistant biotypes and their parent strains. The difference between the growth curves of the pairs of strains (Chart 2) can be correlated with the differences in growth rate of the strains (Chart 3). The curves of the treated resistant biotypes H37RvR and "AR" are both lower than their respective treated sensitive parent strains. The same concentration (9—10 per ml.) of Nocardin gives 50 % inhibition in strain "F" and its biotype "FR". Similarly in Chart 2, Fig. 2, these strains grow at the same rate. The resistant biotype "HR" requires more Nocardin to give 50 % inhibition in growth than the control. This is also consistent with the fact that the resistant biotype grows more rapidly than the streptomycin sensitive biotype (Chart 2, Fig. 3).

CHART 3

THE TUBERCULOSTATIC ACTION OF NOCARDIN (PARTIALLY PURIFIED) ON 4 STREPTOMYCIN RESISTANT AND CONTROL STRAINS



Discussion and Summary

In addition to Nocardin a few other tuberculostatic antibiotics have been described from the same genus. In 1942 Gardner and Chain (7) reported a tuberculostatic antibiotic, "Proactinomycin" from a species of Proactinomyces which by some mycologists are classified among the Nocardia. In 1946 Gause (8) described another tuberculostatic antibiotic, "Litmocidin" from the same group of molds. In 1949 Johnston (9) reported "Mycomycin" from Nocardia acidophilus (n.sp.) as active against the tubercle bacillus. More recently in Switzerland Stoll, Brack and Renz isolated "Nocardamin" from Nocardia Trevisan (10) and gave its chemical formula as $C_9H_{14}O_3N_2$ (11).

Stoll, Brack and Renz (10) state that Nocardamin is characterized by a trimethylene imine (acetidine) ring and by a hydroxylamine grouping. They add that the antibiotic is not identical with any antibiotic known up to this time.

While the chemical nature of Nocardin is as yet unknown, the fact that it inhibits the growth of streptomycin-sensitive strains of tubercle bacilli as readily as the streptomycin resistant biotypes suggests that its chemical structure is different from streptomycin. No direct comparison has as yet been possible between Nocardin and Nocardamin reported in Switzerland.

Since the preparation of the Nocardin fraction by phosphotungstic acid method, the culture medium for the mold has been modified so that the time required to produce a filtrate of equal potency can be reduced from 6 to 3 weeks. In addition the potency of the crude filtrate has been increased by the addition of growth promoting elements to the culture medium. Various methods of purification have been tried in an effort to improve the purity of the product.

The present report has shown that:

1. A stable Nocardin concentrate was obtained by a process involving the adsorption of the active substance on Darco G-60, elution from the adsorbate, elimination of neutral and alkaline alcohol insoluble impurities and finally precipitation with phosphotungstic acid and recovery from the decomposed precipitate. Such a fraction in a concentration of 7 gamma per ml. inhibits the growth of the tubercle bacillus (Strain H37 Rv) fifty per cent.
2. The tuberculostatic activity of this preparation of Nocardin has been tested against four strains of human tubercle bacilli and their streptomycin resistant biotypes. The antibiotic has been found to be equally active against the control strain as against the streptomycin resistant biotypes.
3. Three of the streptomycin resistant biotypes were found to grow at different rates from that of their parent sensitive strains. In another pair both sensitive parent strain and streptomycin resistant biotype grew at the same rate. The amount of drug required to give 50 % inhibition in vitro is directly related to the rate of growth of the strains.
4. A formula which will maintain rapid growth of the tubercle bacillus in submerged culture has been given.

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Immunologic Studies on the Etiologic Agents of North and South American Blastomycosis

I. COMPARISON OF HYPERSENSITIVITY REACTIONS.

by

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(15.10.1952)

For many years South American blastomycosis (1, 2, 3, 4, 5) was confused with coccidioidomycosis and with North American blastomycosis. In the earlier literature the South American disease was thought to be coccidioidal granuloma and the casual organism was identified as *Coccidioides immitis*. This confusion was due to a misinterpretation of the method of reproduction, of the South American fungus as it appeared in infected tissue. In 1929 however, ALMEIDA (6) published the first of a series of papers in which he showed the South American organism to be different from *C. immitis* and (7) proposed the name *Paracoccidioides brasiliensis*. Out of the controversy which followed, there evolved acceptance of these generic differences and the classification proposed by Almeida was recognized.

MOORE (8), in 1938, pointed out the difficulty sometimes encountered in the differentiation of the etiologic agents of North and South American blastomycosis as they appeared in tissue. He found that the South American organism reproduced in tissue not only by multiple budding, but also by single buds which resembled the tissue phase of *Blastomyces dermatitidis*, the etiologic agent of the North American disease. The striking similarity, however, of the fungi of North and South American blastomycosis in culture and in tissue was brought out in the comparative studies by CONANT and HOWELL (9, 10). Conidia, when present in the mycelial phase of the South American fungus, resembled those of *B. dermatitidis*. Also, although single budding predominated in *B. dermatitidis* and multiple budding predominated in the South American organism, both forms were observed in the yeast-like tissue phase of both organisms. The differences noted were said to be of specific rather than of generic importance and the name *Blastomyces brasiliensis* was proposed for the South American fungus, although this suggestion received rather severe criticism from MOORE (10) and WEIDMAN (10). LACAZ (11) also has described *B. dermatitidis* as "fungo este dujos caracteres morfologicoes se assemelham as *Paracoccidioides brasiliensis*." He stated later, 1949 (12), that the differences were not sufficient to separate generically the two organisms and considered, on the grounds of clinical data and microscopic morphology, the two fungi appear to be different species of the same genus.

The extensive work on the morphologic relationship between these two organisms has not been paralleled by similar studies on their antigenic relationship. Since an intradermal reaction to homologous antigens has been established in both North and South American blastomycosis (13, 14), the skin test has been used in this investigation for an immunologic comparison of *B. dermatitidis* and *B. brasiliensis.*

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[1] A part of this investigation was used in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Materials and Methods

Semsitization of guinea pigs. — White guinea pigs, weighing between 400 and 900 grams were determined to be non-sentitive to both *B. dermatitidis* and *B. brasiliensis* by intradermal inoculations with 0.1 ml. of a 1 : 100 dilution by volume of a heat-killed, yeast-phase vaccine of each organism. Each animal was inoculated, intratesticularly and subcutaneously, with 0.05 ml. per 50 gram body weight, of a 1 : 10 dilution of viable, yeast-phase, *B. dermatitidis,* strain 2107 [1]), or *B. brasiliensis,* strain 866.

Skin test antigens — The method used in preparing the yeast-phase, vaccine type antigens was essentially that described by MARTIN and SMITH (13). Saline suspensions in dilutions 1 : 100, 1 : 188 and 1 : 1000, were prepared with both *B. dermatitidis,* 2107, and *B. brasiliensis,* 866. These suspensions were heat-killed at 58° C for one hour and stored at 3 to 6° C. For the comparative study, skin test inoculations were 0.1 ml. in volume. The method used in preparing filtrate antigens was similar to that of EMMONS and his associates (15). Synthetic broth cultures of several strains of *B. dermatitidis* were incubated from 62 to 78 days, Seitz filtered and the filtrate stored at 3 to 6° C. Pooled filtrates from two 71 day old cultures of *B. dermatitidis,* strain 1035, were selected for standardization. *B. brasiliensis* antigens, similarly prepared and stored, were filtrates of cultures 60 to 90 days old and, one, seven months. Preliminary skin tests, on inoculated animals, indicated the filtrate of a 7 month old culture of *B. brasiliensis,* strain 866, to be far superior in antigenic potency. Consequently, this lot was selected for standardization. Ten normal guinea pigs were inoculated intradermally with 0,1 ml. of each lot of undiluted filtrate. None of these control animals reacted to these materials.

Standardization of antigens — The end-point selected for the standardization of the filtrate antigens for skin testing was that concentration of filtrate which, when inoculated intradermally in 0.1 ml. amounts, would produce a positive reaction in 50 per cent of the guinea pigs previously inoculated with the viable, homologous organism. A positive reaction was empirically defined as induration of not less than 5 mm. in diameter or erythema of not less than 10 mm. in diameter. The dilution which represented the 50 per cent end-point (ST50) was calculated according to the method of REED and MUENCH (16). Standardizations were performed when preliminary skin tests showed the sensitivity of the animals to be, at that time, at its maximum. These results are presented in Table I.

For the comparative studies, guinea pigs sensitive to *B. dermatitidis* were inoculated intradermally with 0.1 ml. amounts of three different dilutions of standardized *B. dermatitidis* filtrate and three different dilutions of standardized *B. brasiliensis* filtrate. The dilutions were prepared as follows:

a. Such that 0.1 ml. would react positively in 50 per cent of the animals sensitized to the homologous organism as determined by the standardization procedures-expressed as one ST50.

b. Such that 0.1 ml. would be three times as concentrated as the dilution of (a)-expressed as three ST50.

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[1]) Fungi used in this study were obtained from the culture collection at Duke University, School of Medicine, Dept. of Bacteriology.

c. Such that 0.1 ml. would be ten times as concentrated as the dilution of (a)-expressed as ten ST50.

The same procedure was repeated using animals sensitive to *B. brasiliensis*.

TABLE I. *Standardization of filtrate antigens on experimentally inoculated guinea pigs (positive intradermal reactions expressed in millimeters)*

Animals inoculated with Blastomyces dermatitidis					Animals inoculated with Blastomyces brasiliensis					
	Dilution of unstandardized *Blastomyces dermatitidis* *filtrate*					Dilution of unstandardized *Blastomyces brasiliensis* *filtrate*				
Animal number	1 : 10	1 : 20	1 : 40	1 : 80	Animal number	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64
5	5	10	5	6	1	—	—	—	—	—
15	15	—	—	—	2	16	9	5	7	5
3	15	5	6	5	4	7	10	—	—	—
4	—	—	—	—	5	9	—	—	—	—
12	7	7	—	—	8	25	20	10	6	8
18	14	6	9	11	11	7	—	6	—	6
19	10	10	7	10	15	9	7	6	—	—
16	12	7	5	6	16	9	10	6	—	—
8	—	—	—	—	18	20	15	6	7	—
6	6	9	—	—	20	15	20	11	10	6
9	—	—	—	—	21	15	5	8	10	—
17	—	5	8	10	22	6	8	15	5	—
10	7	6	—	—	23	7	7	6	6	—
14	10	—	6	—	24	17	12	10	7	—
2	5	5	—	—	25	15	11	14	10	—
1	8	10	—	—	26	6	19	—	7	—
					29	18	19	12	17	—
					30	20	14	10	8	—
Number of reactors	12	11	7	6	Number of reactors	17	15	14	12	4
Number of non-reactors	4	5	9	10	Number of non-reactors	1	3	4	6	14
ST50 = 1 : 32.9 dilution					ST50 = 1 : 37 dilution					

Results

Reactions of animals to homologous and heterologous antigens — Cross-reactions were observed among the nineteen animals previously sensitized with *B. dermatitidis* when they were skin tested with the filtrate antigens prepared from cultures of *B. brasiliensis* (Table II). Four (13 per cent) of seventeen animals which reacted to 1 ST50 dose of the homologous filtrate (*B. dermatitidis*) also reacted to the same concentration of the heterologous antigen (*B. brasiliensis*). All nineteen reacted to a ST50 dose of homologous antigen and twelve (63 per cent) of these also reacted to a ST50 dose of heterologous antigen. When the heterologous antigen was increased to 10 ST50 per dose, positive results were obtained in eighteen (95 per cent) of the animals.

Similar results were obtained when yeast-phase vaccine antigens were used to skin test thirteen of these same guinea pigs which had been sensitized with

B. dermatitidis. Ten of the thirteen animals reacted to a 1 : 1000 dilution of *B. dermatitidis* vaccine; two (20 per cent) of these also reacted to a 1 : 1000 dilution of the heterologous *B. brasiliensis* vaccine. When both vaccines were diluted 1 : 188, twelve animals reacted to the homologous antigen and five (42 per cent) of these to the heterologous antigen. Use of a still greater concentration, 1 : 100, produced positive results in all thirteen animals with the homologous vaccine but no further increase in the number of animals reacting to the heterologous antigen.

Cross-reactions also were observed in guinea pigs previously sensitized with *B. brasiliensis* (Table II). Eighteen animals tested with the filtrate antigens gave positive reactions as follows: five reacted to a 1 ST50 dose of the homologous antigen (*B. brasiliensis*), none to a 1 ST50 dose of the heterologous antigen (*B. dermatitidis*); thirteen reacted to a 3 ST50 dose of the homologous antigen and six (46 per cent) of these reacted to a 3 ST50 dose of the heterologous antigen; sixteen reacted to a 10 ST50 does of the homologous antigen and eleven (69 per cent) of these were also positive to a 10 ST50 dose of the heterologous antigen.

TABLE II. *Serologic reactions with sera from immunized rabbits*

Inoculated with Blastomyces dermatitidis				Inoculated with Blastomyces brasiliensis			
Animal Number	Type Antigen	*Antibody Titer* Homologous	Heterologous	Animal Number	Type Antigen	*Antibody Titer* Homologous	Heterologous
2	yeast	1 : 16	1 : 8	9	yeast	1 : 64	1 : 32
	protein	1 : 32	1 : 8		protein	1 : 32	1 : 32
	filtrate	1 : 32	1 : 8		filtrate	1 : 16	1 : 16
17	yeast	1 : 64	1 : 64	15	yeast	1 : 64	1 : 32
	protein	1 : 128	1 : 16		protein	1 : 32	1 : 32
	filtrate	1 : 16	1 : 32		filtrate	1 : 32	1 : 16
19	yeast	1 : 32	1 : 32	14	yeast	1 : 16	1 : 8
	protein	1 : 128	1 : 64		protein	1 : 4	1 : 4
	filtrate	1 : 64	1 : 32		filtrate	1 : 16	1 : 8
18	yeast	1 : 64	1 : 64	16	yeast	1 : 16	1 : 16
	protein	1 : 64	1 : 64		protein	1 : 16	1 : 8
	filtrate	1 : 64	1 : 32		filtrate	1 : 16	1 : 8
20	yeast	1 : 64	1 : 128	21—22	yeast	1 : 64	1 : 32
	protein	1 : 256	1 : 256		protein	1 : 64	1 : 32
	filtratie	1 : 64	1 : 64		filtrate	1 : 32	1 : 16

Nineteen of the *B. brasiliensis* animals also were tested with yeastphase vaccine antigens. Cross-reaction to *B. dermatitidis* was manifest by the following results: five reacted positively to the 1 : 1000 dilution of homologous antigen and none to the 1 : 1000 dilution of heterologous antigen; eighteen reacted to the 1 : 188 dilution of homologous antigen and nine (50 per cent) of these also reacted to the 1 : 188 dilution of the heterologous antigen; all animals reacted to the 1 : 100 dilution of homologous antigen and nine (47 per cent) of these reacted to the same concentration of the heterologous antigen.

None of these antigens, in any concentration employed, produced reactions in ten normal animals.

Reactions of humans to homologous and heterologous antigens — Six patients, proved to be infected with and sensitive to *B. dermatitidis*, were skin tested with a *B. brasiliensis* yeast-phase vaccine antigen. Two of these patients reacted to 0.1 ml. of a 1 : 1000 dilution of the heterologous antigen. The remaining four patients gave negative skin tests with this dilution of antigen but gave positive reactions when inoculated with 0.1 ml. of a 1 : 100 dilution of the same antigen (Table III).

TABLE III. *Cross-reactions in humans infected with blastomyces dermatitidis (positive intradermal reactions expressed in millimeters)*

	Yeast-phase antigen		
	Blastomyces dermatitidis	Blastomyces brasiliensis	
	Dilution		
Patient	1 : 1000	1 : 1000	1 : 100
T.F.	20	4	15
G.C.	25 × 40	10	
E.B.	20	0	7
M.C.	15 × 30	10	10
F.M.	18	4	20
J.H.	positive1)	0	20

1) Reaction not measured.

Discussion

The results obtained in this study indicate that *B. brasiliensis* and *B. dermatitidis* possess a similar antigenic substance, or substances, which evokes a state of hypersensitivity in inoculated animals and infected individuals which is responsible for the cross-sensitivity reactions observed. Guinea pigs, inoculated with *B. brasiliensis* and found to be sensitive to a filtrate antigen of the homologous organism (*B. brasiliensis*), reacted when tested intradermally with the filtrate antigen of the heterologous organism (*B. dermatitidis*). Conversely, animals inoculated with *B. dermatitidis* and found to be sensitive to a filtrate antigen of the homologous organism (*B. dermatitidis*), reacted when tested intradermally with a filtrate antigen of the heterologous organism (*B. brasiliensis*). With some of the animals it was necessary to use a more concentrated antigen to detect this crossreaction. Normal animals, however, did not react to these filtrate antigens. Other investigators (17) have shown that an increased concentration of antigen may be necessary to detect sensitivity in patients with a fungus disease.

An antigenic relationship between these two organisms also was demonstrated by cross-reactions in properly prepared animals to intradermal inoculations of the yeast-phase antigens. It was found however that the incidence of cross-reactions was not so great with the yeast-phase antigens as when the filtrate antigens were used. This could be due to a portion of the antigens of a cell suspension being inaccessibly bound within the cell whereas antigens in a filtrate are probably in a free state. If those antigens bound within the cell were of the substance which the two organisms have in common the quantity of cross-reacting antigens available for action would be reduced. Accordingly the incidence of cross-reaction would be less than that elicited by the filtrate antigens. For this reason the two types of antigens may not be comparable. It also was noted that an increased concentration of the heterologous filtrate antigen elicited a slightly greater increase in cross-reaction than was produced when the yeast-phase antigens were proportionately concentrated. However the concentration of a yeast-phase antigen from 1 : 1000 to 1 : 100 and a filtrate antigen from 1 ST50 to 10 ST50 in order to increase potencies may not represent a comparable increase in the active principal responsible for the cross-reaction.

The evidence thus far presented in support of an immunologic relationship between *B. dermatitidis* and *B. brasiliensis* by animal experimentation was supported by the results obtained with human cases of North American blastomycosis. Unfortunately, for purposes of this investigation, few patients were available for study. Of those available, not all responded with a positive skin test to an inoculation of homologous antigens and were, therefore, unsuitable for studies with heterologous antigens. Two of the six patients who were allergic to *B. dermatitidis* yeast-phase antigen, however, reacted to the same concentration of the *B. brasiliensis* yeast-phase antigen. By increasing the concentration of the heterologous antigen, the incidence of cross-reaction was increased to include the remaining four patients. From the results obtained it could be concluded either that the degree of cross-reactions to these materials is slight or that the antigens used were not comparable.

No human cases of South American blastomycosis were available for study in this country. Lacaz (14), in Brazil, skin tested twenty-five patients with paracoccidioidin (prepared from *B. brasiliensis*), blastomycetin and coccidioidin. Eighteen patients reacted to paracoccidioidin, five of the eighteen reacted to blastomycetin and none reacted to coccidioidin.

Lacaz stated, "Doentes de blastomicose sul-americana podem reagir positiveamente à blastomicetina, se bem que a frequencia de reações positivas nãõ seja elevado (reações de grupo)." A greater concentration of heterologous skin test material would, no doubt, have increased the incidence of cross-reactions among these patients. It is also of interest that Versiani (19) found 28 patients, hypersensitive to *B. brasiliensis* skin test material, were negative when tested with coccidioidin, 1 : 1000 and 1 : 100.

Summary

This study presents an antigenic comparison of the fungi, *B. brasiliensis* and *B. dermatitidis*, by intradermal reactions in animals and humans.

Guinea pigs, hypersensitive to the homologous infecting fungus, reacted when skin tested with standardized broth filtrates and yeast-phase vaccines of the heterologous organism. Similar results were obtained in the limited number of human cases of North American blastomycosis when the yeast-phase vaccines were employed.

A statistical method, the 50 per cent end-point, was utilized for standardizing broth filtrates of fungi to be employed as skin test antigens.

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Immunologic Studies on the Etiologic Agents of North and South American Blastomycosis

II. COMPARISON OF SEROLOGIC REACTIONS.

by

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A preceding report (1) has demonstrated an immunologic relationship between *Blastomyces dermatitidis* and *B. brasiliensis* by means of hypersensitivity reactions. Since reports have been made concerning the complement fixing antibody in the sera of patients with North and South American blastomycosis (2, 3, 4) and this antibody and its detection by the complement. fixation test in animals experimentally inoculated with *B. dermatitidis* or *B. brasiliensis* (5, 6) also has been reported, the complement fixation test has been used in the following comparative study of *B. dermatitidis* and *B. brasiliensis* to investigate further the possible antigenic relationship between these two organisms.

Materials and Methods

Serologic test. — The EP50 complement fixation test as described by Kent and co-workers (7, 8, 9, 10, 11) was used[2]). Human sera were inactivated at 56° C for thirty minutes and rabbit sera at 61° C for the same interval of time. Before beginning the actual test, sera were diluted 1 : 3 and from this dilution progressive two-fold dilutions, usually 1 : 1 through 1 : 64, were made. Titers have been expressed in terms of these last mentioned dilutions, that is 1 : 1, 1 : 2, 1 : 4, *et cetera,* and the preliminary dilution of 1 : 3 has been ignored. The degree of reaction was determined by comparison with standards of 0, 25, 50, 75, 90, and 100 per cent hemolysis to simulate, respectively, ++++, +++, ++, +, +, and — fixation of complement.

Antigens. — Three types of antigens were used. Both the filtrate of mycelial-phase broth cultures and saline suspensions of yeast-phase cultures were employed. The methods of preparation were similar to those reported for preparing skin test antigens (1). Lots were selected on the basis of antigenic potency and minimal, or absence of, anticomplementary activity. It was necessary, however, to prepare several lots of the yeast-phase antigens in order to find one with a minimal anticomplementary activity which would permit its use in the complement fixation test. This was especially true of preparations made from *B. brasiliensis.*

In addition, a "purified" protein antigen was prepared from each organism by disintegrating the yeast-phase cells with sonic vibrations and then removing the protein by precipitation with ammonium sulphate. The procedure differed from that of MARTIN (12) primarly in the selection of a time interval for sonic vibration of the cell suspensions in order to extract a maximum of undenatured protein. The optimal time was determined by vibrating sample batches of yeast-phase suspensions in a 9KC magneto striction oscillator [1]) for varying periods of time: 0, 5, 10, 15, 20, 45, 60, 120, and 180 minutes. These crude samples were then used as antigens, at the optimal antigen dilution, in the complement fixation test with homologous antiserum in order to determine which exposure time most greatly increased the antigenic sensitivity. As the time of exposure to sonic vibrations increased, the antigenicity of the material also increased until a peak was reached beyond which further increase in time of vibration produced no further increase in antigenicity. Moreover, a continuous increase in exposure time resulted in a slight decrease in antigenic activity. Thirty minutes was selected as the time interval for exposing *B. dermatitidis* yeast-phase cells to sonic vibrations.

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[1]) A part of this investigation was used in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2]) Complement, hemolysion and cells were standardized by Dr. Kent during the course of his studies and quantities necessary for this investigation were generously supplied by him.

The optimal time for the sonic vibration of *B. brasiliensis* yeast-phase cells was similarly determined. Because of interference by the anticomplementary activity of these cell suspensions in the lower dilutions, however, true optimal antigen concentrations could not be determined. Instead, sonic vibrated samples were compared entirely on the basis of the highest dilution which produced complement fixation reaction in the highest dilution of anti-serum. A maximum of antigenic material was released from the *B. brasiliensis* cells after five minutes exposure to sonic vibrations and no decrease was noted after sixty minutes. Ten minutes was selected as the interval of time for sonic vibration of this organism preliminary to removing the protein.

At the concentration optimal for the complement fixation reaction, the final "purified" protein antigens were negative when tested with Molisch reagent.

In order to assume maximum reaction of antigen and antibody in a given serum, the optimal concentration of each antigen to be used in the comparative studies was determined by testing with homologous antiserum from rabbits. Subsequent tests, however, indicated that this antigen concentration was not always satisfactory for testing human sera. Therefore, in certain instances, a different dilution of antigen was used to titer human sera.

Antisera. — The sera of patients with North or South American blastomycosis [2]) and the sera of immunized, non-infected rabbits were used as a source of antibodies. Rabbits were inoculated with sonic disintegrated yeast-phase cells of either *B. dermititidis*, strain 2107, or *B. brasiliensis*, strain 866, as follows: day 1, 1 ml. of 0.5 per cent cells by volume; day 2, 1 ml. of 0.5 per cent cells; day 3, 1 ml. of 5 per cent cells; day 4, 2 ml. of 5 per cent cells; day 10, 2 ml. of 5 per cent cells; day 16, 3 ml. of 5 per cent cells; day 22, 4 ml. of 5 per cent cells. All animals except one were exsanguinated on day 26. One animal, which was moribund, was exsanguinated on day 23.

RESULTS OF STUDY

Homologous and heterologous reactions with animal sera: Each serum was tested with both homologous and heterologous antigens for complement fixing antibodies. Yeast-phase, filtrate and "purified" protein antigens were used. The results are shown in table I. In this table it can be seen that marked cross-reaction was observed in all animal sera using all types of antigens.

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[1]) Type R 22—3, available from Raytheon Manufacturing Company, Waltham, Massachusetts.

[2]) Thanks are expressed to Dr. C. S. LACAZ, University of São Paulo, Brazil, and to Prof. L. BOGLIOLO, University of Minas Gerais, Brazil, for supplying sera from patients with South American blastomycosis.

Results of Study

In most instances, the use of heterologous antigens in the complement fixation test produced antibody titers as high, or almost as high, as the titers shown by use of the homologous antigens. In two instances, rabbit sera 17 and 20, showed slightly higher titers with the heterologous antigen prepared from the yeast-phase. Certain of the results, however, differed from this observation in that the degree of cross-reaction was not so high. This is exemplified by the serum of rabbit 2 which was immunized against *B. dermatitidis*; the antibody titer with the homologous protein and filtrate antigens was 1 : 32 whereas the titer when using the heterologous antigens of the same type was only 1 : 8.

Homologous and heterologous reactions with human sera: Sera obtained from individuals infected with *B. dermatitidis* or *B. brasilienses* were tested in the same manner as the animal sera. In some instances, however, the quantities of sera available were not sufficient to permit the performance of all tests. As indicated in table II, eleven of twenty specimens of sera from cases of North American blastomycosis fixed complement with the homologous antigens and two of these, 72 and GC, cross-reacted at a low titer with heterologous antigens.

Sera from eleven cases of South American blastomycosis fixed complement with their homologous antigens. Cross-reactions were observed in five of these sera with one or more types of the heterologous antigens. Detailed results are shown in table II and in these the varying degrees of cross-reaction may be seen. Not all of the sera showing high complement fixing antibody titers with the homologous antigens, cross-reacted to high titers with the heterologous antigens and some high titered sera showed no cross-reaction. For example, the serum of South American blastomycosis patient, AJS, was found to possess a high antibody titer (1 : 256) with the homologous antigens yet no cross-reaction was observed when this serum was tested with the heterologous *B. dermatitidis* antigens. The serum of South American blastomycosis patient, RT, which also possessed a high antibody titer (1 : 128) when tested with the homologous antigens, showed a marked cross-reaction (1 : 16 with the filtrate antigen) to the heterologous *B. dermatitidis* antigen. Patient, BHII, also infected with *B. brasiliensis*, possessed a relatively low titer (1 : 8) with the homologous antigens, yet showed a definite cross-reaction (titer 1 : 2) with the heterologous antigens.

Complement fixation tests with sera from ten normal individuals and eighteen tuberculosis patients were made using the antigens of both fungi. One antigen, the *B. brasiliensis* yeast-phase, reacted with one serum through a dilution of 1 : 8.

Discussion of Serologic Reactions

Evidence has been presented by means of comparative complement fixation studies that an antigenic relationship exists between *B. dermatitidis* and *B. brasiliensis*. Although the cross-reactions in the complement fixation tests are not so marked as the cross-reaction of hypersensitivity (1), complement was fixed with heterologous antigens in the presence of antibodies both human and animal antisera. Whether or not the relation observed is as great as, or less than, the relation which may exist between either of these two organisms and other fungi was not determined.

The most striking observation made was the consistent and high degree of cross-reactions obtained with rabbit antisera and the erratic results obtained with human antisera. Cross-reaction was observed with only two *B. dermatitidis* human sera, patients 72 and GC, when tested with heterologous antigens. Such a finding, however, was not surprising since titers of homologous antibodies were low. Apparently, antibody stimulation by *B. dermatitidis* is poor and when the homologous antibodies are few, antibodies corresponding to the heterologous antigens are present in such small quantities that they could not be detected by the methods used. On the other hand, the higher titers obtained with homologous antigens and sera for human cases of South American blastomycosis showed *B. brasiliensis* to be a more efficient stimulator for antibody formation and, therefore, a greater incidence of cross-reaction with the heterologous *B. dermatitidis* antigens resulted. In all cases, however, the sera which gave the highest titer of antibodies when tested with homologous antigens did not necessarily give a correspondingly high degree of cross-reaction with heterologous antigens. Since repeated tests with these sera gave the same degree of fixation, this discrepancy is known not to be a fault of the test methods used but an inherent quality of the sera.

Antigens obtained from *B. dermatitidis* and *B. brasiliensis* also were used in complement fixation tests with sera from ten normal individuals and with sera from eighteen patients with extensive tuberculosis. As only one serum, from a patient with tuberculosis, showed fixation in low titer in the presence of one fungus antigen, the yeast-phase of *B. brasiliensis*, the antigens were considered to be specific.

While sera from humans infected with *B. dermatitidis* gave consistently low complement fixation titers with their homologous antigens, those sera obtained from rabbits immunized with this fungus gave high complement fixation and cross-reaction titers. Since these rabbits were immunized with yeast-phase cultures grown on an agar medium and agar is known to be antigenic (13), it was felt that traces of agar might have been responsible for some of the antibody response detected in the sera when the same yeast-phase antigen was used in the tests. It was found, however, that the filtrate antigen obtained from a synthetic liquid medium in which *B. dermatitidis* was grown gave correspondingly high titers and cross-reactions in complement fixation tests with the same sera. The high antibody content and cross- reaction of these sera, therefore, could not have been due to traces of agar but, rather was a response by the animal to repeated, massive doses of sonic disintegrated cells. By the same reasoning, the high degree of cross-reaction observed using the sera of rabbits immunized against *B. brasiliensis* could not be due to the presence of agar in the immunizing antigens.

In such a comparative study, it was necessary to determine the optimal dilution of the antigens to be compared in order to assume maximum reaction of antigen and antibody in a given serum. It was found in these studies, however, that when an optimal dilution of antigen for measuring antibodies in rabbit serum had been determined, this same dilution was not optimal in tests with human serum. These experimental studies point out the danger of assuming standardization of an antigen by its behavior in a particular type of serum.

The treatment of yeast-phase cells to sonic vibrations clearly demonstrated the difference in fragility of the yeast cells of these two fungi. After five minutes vibration, *B. brasiliensis* yeast cells disintegrated and the supernate of the suspension was opaque. On the other hand, it required twenty minutes vibration to produce this same effect with yeast cell suspensions of *B. dermatitidis*. This observation can be correlated with the morphologic characteristics of these organisms. It has been noted in these studies and by MACKINNON AND VINELLI (14) that the yeast cells of B. brasiliensis have a thin wall as compared with that of *B. dermatitidis*. Furthermore, the multiple budding of the former may contribute materialy to the fragility of its yeast cell.

Summary

This study presents an immunologic comparison of the fungi, *B. brasiliensis* and *B. dermatitidis*, by serologic reactions in animals and in humans.

The complement fixation test was used to test the sera of rabbits with various homologous and heterologous antigens of the two fungi: yeast-phase, broth filtrates of the mycelial phase, and "purified" protein. A definite cross-reactivity was observed.

Sera from humans infected with *B. dermatitidis* or *B. brasiliensis* also were tested by complement fixation reaction with homologous and heterologous antigens: yeast-phase, broth filtrates of the mycleial phase, and "purified" protein. Two of nine sera from cases of North American blastomycosis, giving positive reactions with the homologous antigens, cross-reacted at a low titer with the heterologous antigens. Five of eleven sera, positive with *B. brasiliensis* antigens, reacted with one or more types of *B. dermatitidis* antigens. There was no correlation between titer obtained with the homologous antigens and that obtained with the heterologous antigens.

Immunization of rabbits was best achieved by use of massive doses of sonic-vibrated yeast-phase cell suspensions.

Data were presented indicating the greater fragility of *B. brasiliensis* yeast-phase cells as compared with *B. dermatitidis* cells of the same phase. Evidence was obtained by subjecting cells to sonic vibrations.

TABLE I. *Serologic reactions with sera from patients with blastomycosis*

Infected with Blastomyces dermatitidis				Infected with Blastomyces brasiliensis			
		Antibody Titer				Antibody Titer	
Patient	Type Antigen	Homologous	Heterologous	Patient	Type Antigen	Homologous	Heterologous
AG	yeast	1 : 32	neg	AJS	yeast	1 : 4	neg
	protein	1 : 32	neg		protein	1 : 512	neg
	filtrate	1 : 32	neg		filtrate	1 : 512	neg
EB	yeast	1 : 32	neg	AJV	yeast	neg	neg
	protein	1 : 32	neg		protein	1 : 256	neg
	filtrate	1 : 32	neg		filtrate	1 : 256	1 : 2
GC	yeast	1 : 8	1 : 2	MC	protein	1 : 128	neg
	protein	1 : 8	neg		filtrate	1 : 128	neg
	filtrate	1 : 8	neg				
72	yeast	1 : 16	—	TZ	yeast	1 : 64	neg
	protein	1 : 16	1 : 2		protein	1 : 32	neg
	filtrate	1 : 16	1 : 1		filtrate	1 : 128	neg
41	yeast	1 : 4		AJ	yeast	1 : 16	neg
	protein	1 : 16	neg		protein	1 : 8	neg
	filtrate	1 : 16	neg		filtrate	1 : 128	neg
P	yeast	1 : 4		RT	yeast	1 : 32	1 : 2
	protein	1 : 8	neg		protein	1 : 16	1 : 1
	filtrate	1 : 8	neg		filtrate	1 : 128	1 : 16
62	yeast	1 : 16	neg	BHI	yeast	1 : 64	1 : 1
	protein	1 : 16	neg		protein	1 : 64	neg
	filtrate	1 : 16	neg		filtrate	1 : 32	1 : 2
EM	yeast	neg	neg	FSO	yeast	1 : 8	neg
	protein	neg	neg		protein	1 : 64	neg
	filtrate	1 : 8	neg		filtrate	1 : 64	1 : 32
56	yeast	1 : 4		BHII	yeast	1 : 8	1 : 2
	protein	1 : 4	neg		protein	1 : 4	1 : 1
	filtrate	1 : 4	neg		filtrate	1 : 8	1 : 1
				JC	yeast	1 : 1	
					protein	1 : 8	neg
					filtrate	1 : 8	neg
				LD	yeast	1 : 8	neg
					protein	1 : 4	neg
					filtrate	1 : 4	neg

TABLE II. *Cross-reactions in experimentally inoculated guinea pigs (positive intradermal reaction expressed in millimeters)*

	Animals inoculated with Blastomyces dermatitidis											
	Filtrate antigens						Yeast-phase antigens [1])					
	Blastomyces dermatitidis			Blastomyces brasiliensis			Blastomyces dermatitidis			Blastomyces brasiliensis		
	Number of ST50 doses						Dilution					
	10	3	1	10	3	1	1:100	1:188	1:1000	1:100	1:188	1:1000
18	17	12	9	20	11	13						
19	10	15	7	9	—	—	17	15	15	5	—	5
16	12	5	7	9	—	5	14	14	13	5	—	5
17	20	12	—	11	—	—	15	14	5	—	5	—
31	15	10	10	9	7	9	10	10	8	—	—	—
30	17	10	10	7	8	—	13	10	10	—	7	—
28							8	—	5	7	—	—
29	15	12	8	23	10	10	13	10	11	6	5	—
34	11	8	8	7	5	—	9	9	—	—	—	—
33	12	10	9	7	7	—	11	9	9	9	5	—
32	15	11	11	7	5	—	10	9	—	—	—	—
35	10	17	15	8	7	10	8	10	8	—	—	—
27	15	10	6	6	—	—						
24	19	10	10	6	—	—						
25	15	11	10	—	—	—						
26	20	20	20	11	8	—						
21	22	12	11	11	5	—	10	14	12	—	—	—
20	11	12	11	8	9	6						
22	12	13	8	6	—	—	9	10	5	7	6	—
23	12	8	—	15	9							
	Number of reactors			Number of cross-reactors			Number of reactors			Number of cross-reactors		
	19	19	17	18	12	4	13	12	10	5	5	2

	Animals inoculated with Blastomyces brasiliensis											
	Filtrate antigens						Yeast-phase antigens [1])					
	Blastomyces brasiliensis			Blastomyces dermatitidis			Blastomyces brasiliensis			Blastomyces dermatitidis		
	Number of ST50 doses						Dilution					
	10	3	1	10	3	1	1:100	1:188	1:1000	1:100	1:188	1:1000
1	15	9	—	10	—	—	10	9	5	7	5	—
2	10	9	6	9	6	—	10	9	5	7	7	—
4	18	5	—	7	—	—	7	—	—	—	—	—
5	—	—	—	—	—	—	8	9	5	—	5	—
7	17	11	—	23	20	10	5	7	—	—	—	—
8	20	—	—	—	—	—	5	5	—	—	—	—
11	5	—	—	—	—	—	9	7	—	6	—	—
15	—	—	—	—	—	—	8	8	—	—	—	—
16	13	5	—	7	8	—	6	5	—	5	—	—
18	15	5	—	10	—	—	5	5	—	—	—	—
20	30	12	—	—	—	—	10	7	—	6	7	—
21	20	12	—	10	5	—	6	5	—	—	5	—
22	20	7	—	—	—	—	5	—	—	—	—	—
24	20	9	5	10	6	—	8	7	5	7	7	—
25	22	13	7	13	10	—	11	10	—	7	5	—
26	20	12	10	—	—	—	8	8	—	—	—	—
28							6	5	5	—	6	—
29	10	—	—	8	—	—	7	6	—	7	7	—
30	9	5	—	5	—	—	8	8	—	5	—	—
	Number of reactors			Number of cross-reactors			Number of reactors			Number of cross-reactors		
	16	13	5	11	6	0	19	18	5	9	9	0

[1]) Skin tests with yeast-phase antigens were not performed concurrently with those using the filtrate antigens.

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The Effect of Cortisone on the Course of Systemic Moniliasis in Mice*

I. The Efficacious Effect of Cortisone for Severe Infections

by

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Experimental studies by Scherr (1951), Scherr and Weaver (1951), and Scherr (unpublished data) have shown that certain gonadotropic hormones significantly reduce the severity of systemic moniliasis in mice and in some cases free the animals of any pathological condition, as far as can be discerned by macroscopic examination at autopsy. Since Sohval and Soffer (1951) had demonstrated that treatment with cortisone may increase the titre of urinary gonadotropin, it was felt pertinent to study the effect of cortisone on *Candida*-infected mice.

Numerous workers have already reported that cortisone enhances the severity of infections such as brucellosis (Abernathy, 1951), tuberculosis (Freeman *et al.*, 1950; Michael, 1950), streptococcal and pneumococcal infections (Glaser *et al.*, 1951; Thomas *et al.*, 1951), virus infections (Kilbourne, 1951a, 1951b; Kligman *et al.*, 1951), mycotic infections (Kligman *et al.*, 1951; Redaelli *et al.*, 1951; Syverton *et al.*, 1952), syphilis (Turner, 1951), and *Micrococcus* infections (Kligman *et al.*, 1951). (See also Editorials, 1951a, 1951b).

Cortisone may not only decrease the host's resistance to infection, but may reactivate dormant infections and also evoke a pathological response due to organisms usually present in the body as commensals (Editorial, 1951b; Selye, 1951).

Roth *et al.* (1952) and Syverton *et al.* (1952) showed that cortisone enhances the severity of infections induced by *Candida albicans* and that this enhanced severity can be still further increased by the simultaneous use of total body X-irradiation.

By maintaining mice at various environmental temperatures Scherr (1952) showed that an increased temperature (37° C) increased the toxicity of cortisone for mice, that this increased toxicity was more pronounced in male than in female animals, and that a cold temperature (—5° to 7° C) completely reversed the toxic effects of cortisone for both sexes. This enhanced toxicity for male animals was also reported by Ingle (1939) for rats inoculated with "cortin".

A high environmental temperature also markedly enhances the severity of monilial infection in mice (Scherr, 1953b). Thus, for example, a 50 % mortality results when infected mice are kept at 15°—20° C or 30° C for 30 days, but a similar group kept at 37° C shows a 100 % mortality after only 9 days.

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Because of these temperature effects, experimental studies similar to those described here require either temperature controlled animal quarters or infected, untreated and uninfected, treated groups of controls run concomitantly with each experiment. The work reported here utilized the latter method to assess the factor of environmental temperature. The use of these and other controls resulted in data which supports the contention that cortisone, under certain conditions reduces significantly the severity of infection due to *C. albicans.*

Materials and Methods

The mice used were a white Swiss strain, 18 to 22 grams, purchased from R. F. Beyer & Son, Billings, Missouri, All animals received tap water and Purina show pellets *ad libitum,* under both normal and experimental conditions.

C. albicans strain 171D has been described in a previous paper (Scherr, 1953a).

The medium used was a broth employed by Reilly, Harris, and Waksman (1947). Its composition is glucose, 1.0 %; peptone, 0.5 %; beef extract, 0.5 %; NaCl, 0.5 %; distilled water. Thirty ml of broth contained in a 125 ml erlenmeyer was loop-inoculated with *C. albicans* 171D and incubated at 25° C. The centrifuged pellet from such a 48 hr culture was suspended in 16 ml of sterile saline. To procure a more severe infection 5 % mucin, prepared according to Campbell and Saslaw (1950), was substituted for the saline. Mice were infected intraperitoneally with 0.4 ml of either suspension.

Treatment with cortisone (cortisone acetate, Merck) was instituted 48 hours after infection and was administred intramuscularly into the quadruceps femoris in 0.05 and 0.1 ml amounts (0.25 and 0.5 mg cortisone, respectively). Inoculations were repeated every 48 hours on alternate sides of the animal. The aqueous vehicle in which the cortisone is suspended was inoculated in 0.05 and 0.1 ml amounts on the same schedule. Reference in this report to the use of cortisone is to cortisone suspended in aqueous vehicle; the term aqueous vehicle refers to the saline solution containing suspending agents (polyoxyethylene sorbitan monooleate and sodium carboxymethylcellulose) and 1.5 % benzyl alcohol (Aqueous vehicle No. 1, Merck).

The degree of dissemination of moniliasis was gauged from the number, size, and distribution of the lesions. As a rule the condition of the kidneys is an excellent indicator of the severity of the infection. The degree of dissemination was assigned a numerical value, as follows:

0	no visible pathology
1	few, isolated lesions
2	frequent lesions
3	many lesions
4	marked dissemination of lesions
5	very marked dissemination

If the animal died, presumably as a result of *Candida* dissemination, prior to the end of the treatment period, a value 1 was added to the base value 5 for every day prior to the end of the experiment. For example, if an animal died 3 days before the experiment culminated, the dissemination value was designated by the number 8. Thus, the dissemination values recorded here are a measure of both severity of infection and mortality rate. No dissemination values are given for those animals which were found mutilated or were killed accidentally; they are represented by a dash (—) in the various tables. In all cases 10 mice composed an experimental group.

The duration of the experiment was measured from the day the animals were infected to the day the survivors were autopsied; the periods varied and are recorded in the individual experiments.

Where statistical evaluation of the data was indicated, the method of *t* was employed; P values smaller than 0.05 were considered significant. Because such animal experiments result in a very wide range in dissemination values within an experimental group, the standard deviation from the mean is usually large, thus imposing a severe burden on the data before a P value smaller than 0.05 can result. Therefore, P values less than 0.05 indicate excellent statistical validity and values only slightly greater than 0.05 might indicate a trend.

Results

Table 1 records the data for male and female groups of infected mice treated with cortisone. The duration of the experiment was 29 days. The mean dissemination for infected, untreated males was 1.8; this value was enhanced to a mean value of 11.6 by cortisone treatment. However, this mean (11.6) is comparable to that found in the uninfected controls similarly treated with cortisone (12.3). A duplicate experiment with female mice gave equivalent results (table 1). The dissemination value of 3.0 for infected, untreated female mice was increased to 12.5 by this series of cortisone inoculations, but 12.5 is comparable to the value of 8.5 found for uninfected female mice similarly treated.

This experiment was duplicated with the aqueous vehicle in which cortisone is suspended instead of the cortsione; the data are recorded in table 2. Since the tests with the aqueous vehicle and the cortisone were run simultaneously, the controls of infected, untreated male and female animals are the same in both experiments. There were no mortalities among the infected, untreated male animals before the end of the experiment, and the mean dissemination value was 1.8. Treatment with aqueous vehicle increased this value to 9.8, with a 50 % mortality. This enhanced severity of infection cannot be unequivocably attributed to the toxic effects of the aqueous vehicle, as evidenced by 100 % survival of the uninfected, treated group of mice. The female mice similarly treated showed no such enhanced dissemination as found in the male animals. The dissemination values of infected, untreated and infected, treated groups of animals were 3.0 and 4, respectively. Control female mice treated similarly with the aqueous vehicle resulted in 100 % survivors.

The above experiments were repeated but instead of a relatively mild infection a very severe one was induced in male and female mice by inoculating the same numbers of cells suspended in 5 % mucin. The effect of the schedule of cortisone treatment described above on these animals is recorded in table 3. The mean dissemination value of 20.5 for infected, untreated male mice was reduced to 11.6 by treatment with cortisone; here again 11.6 appears to represent a limit governed by the toxicity of cortisone, since uninfected, treated controls showed a value of 12.3. This marked reduction in severity of infection was not manifest in the female mice similarly treated. Thus, values for infected, untreated; infected, treated; and uninfected, treated groups of female mice were 19.5, 25.9, and 8.5 respectively.

A replication of this experiment using aqueous vehicle instead of cortisone

TABLE 1. *Effect of Cortisone Acetate on Mice Systemically Infected with Candida albicans 171D*

(Duration of experiment — 29 days)

Legend	Dissemination		
Infected, untreated (males)	—	Mean	1.8
	—	Average deviation from mean	1.5
	0	Stand. deviation from mean	1.75
	0		
	0		
	1		
	2		
	2		
	4		
	5		
Infected, treated with 0.50 mg intramuscularly every 48 hrs starting 2 days after infection (males)	1	Mean	11.6
	1	Av. dev. from mean	8.8
	3	Stand. dev. from mean	10.0
	3		
	7	*t* for infected, treated males compared to infected, untreated males	—2.59
	11		
	16		
	19	Degrees of freedom	16
	24	P	.02—.01
	31		
Uninfected, treated as above (males)	0	Mean	12.3
	0	Av. dev. from mean	13.4
	0	Stand. dev. from mean	13.6
	0		
	1		
	6		
	29		
	29		
	29		
	29		
Infected, untreated (females)	—	Mean	3.0
	—	Av. dev. from mean	5.0
	0	Stand. dev. from mean	7.6
	0		
	0		
	0		
	0		
	0		
	1		
	23		
Infected, treated with 0.50 mg intramuscularly every 48 hrs starting 2 days	2	Mean	12.5
	3	Av. dev. from mean	8.3
	3	Stand. dev. from mean	8.5
	5		
	8	*t* for infected, treated females compared to infected, un-	
	19		

Table 1 (continued).

Legend	Dissemination		
after infection (females)	20	treated females	2.3
	20	Degrees of freedom	16
	22	P	.05—.02
	23		
Uninfected, treated as above (females)	0	Mean	8.5
	0	Av. dev. from mean	10.5
	0	Stand. dev. from mean	11.83
	0		
	0		
	0		
	7		
	20		
	29		
	29		

TABLE 2. *Effect of Aqueous Vehicle of Cortisone Acetate on Mice Systemically Infected with Candida Albicans* 171*D*

(*Duration of experiment — 29 days*)

Legend	Dissemination		
Infected, untreated (males)	Same as in Table 1	Mean	1.8
Infected, treated with 0.1 ml aqueous vehicle of cortisone acetate every 48 hrs starting 2 days after infection (males)	0	Mean	9.8
	0	Av. dev. from mean	9.4
	0	Stand. dev. from mean	10.9
	0		
	2	*t* for infected, treated males compared to infected, untreated males	—.195
	12		
	12		
	15	Degrees of freedom	16
	27	P	.10—.05
	30		
	—		
Uninfected, treated as above (males)	0		
	0		
	0		
	0		
	0		
	0		
	0		
	0		
	0		
Infected, untreated (females)	Same as in Table 1	Mean	3.0
Infected, treated	0	Mean	4.0

Table 2 (continued).

Legend	Dissemination		
with 0.1 ml aqueous vehicle of cortisone acetate every 48 hrs starting 2 days after infection (females)	0	Av. dev. from mean	5.2
	0	Stand. dev. from mean	6.78
	0		
	1	t for infected, treated females compared to infected, untreated females	—.28
	1		
	2		
	2		
	14	Degrees of freedom	16
	20	P	.80—.70
Uninfected, treated as above (females)	0		
	0		
	0		
	0		
	0		
	0		
	0		
	0		
	0		
	0		

(table 4) resulted in an enhancement of the dissemination value, 20.5 to 30.4, in the male mice, but not in the female mice. In the latter case the values for infected, untreated and infected, treated mice were 19.5 and 17.7, respectively.

Discussion

The salient features one may infer from the above data, which have been confirmed by a number of other experiments (SCHERR, unpublished data), are as follows:

Treatment of a mild monilial infection in mice with cortisone, using doses and regimen of inoculations which are toxic for normal mice, enhances the severity of the infection in both male and female animals. However, at least under the conditions of these experiments, this enhancement is no greater than that which may be attributed to the toxicity of the cortisone. Thus we have not been able to demonstrate a synergistic deleterious effect of cortisone on monilial involvement.

The use of the aqueous vehicle alone, at concentrations which displayed no untoward effect on normal mice of either sex, enhanced the severity of the disease in infected animals. This enhancement was much more pronounced in male than in female animals and was found to occur whether a mild or severe infection had been induced.

Since the aqueous vehicle enhances the severity of infection in infected mice and shows no toxic effects on normal mice, at least as could be discerned by macroscopic examination at autopsy, it might be expected that using cortisone (in this aqueous vehicle) for infected mice would result in an increased severity of infection much greater than either that level due to the toxicity of cortisone on normal mice or the level found with the aqueous vehicle alone on infected mice. However, this is not the case at all. One possible explanation might be that cortisone has at least two separate effects:

TABLE 3. *Effect of Cortisone Acetate on Mice Systematically Infected with Candida albicans 171D Suspended in Mucin*

(Duration of experiment — 29 days)

Legend	Dissemination		
Infected, untreated (males)	1	Mean	20.5
	2	Av. dev. from mean	11.3
	2	Stand. dev. from mean	12.6
	22		
	27		
	29		
	29		
	31		
	31		
	31		
Infected, treated with 0.50 mg intramuscularly every 48 hrs starting 2 days after infection (males)	1	Mean	11.6
	1	Av. dev. from mean	8.7
	3	Stand. dev. from mean	10.0
	3		
	7	*t* for infected, treated males compared to infected, untreated males	1.66
	11		
	16		
	19		
	24	Degrees of freedom	.20—.10
	31		
Uninfected, treated as above (males)	Same as in Table 1	Mean	12.3
		t for uninfected, treated males compared to infected, treated males	0.12
		Degrees of freedom	18
		P	.90
Infected, untreated (females	2	Mean	19.5
	2	Av. dev. from mean	9.9
	5	Stand. dev. from mean	11.7
	20		
	20		
	22		
	31		
	31		
	31		
	31		
Infected, treated with 0.50 mg intramuscularly every 48 hrs starting 2 days after infection (females)	16	Mean	25.9
	19	Av. dev. from mean	4.5
	22	Stand. dev. from mean	5.0
	24		
	28	*t* for infected, treated females compared to infected, untreated females	1.42
	30		
	30		
	30		

Table 3 (continued).

Legend	Dissemination		
	30	Degrees of freedom	18
	30	P	.20—.10
Uninfected, treated as above (females)	Same as in Table 1	Mean	8.5
		t for uninfected, treated females compared to infected, treated females	4.05
		Degrees of freedom	18.0
		P	.001

TABLE 4. *Effect of Aqueous Vehicle of Cortisone on Mice Systematically Infected with Candida albicans* 171*D Suspended in Mucin*
(Duration of experiment — 29 days)

Legend	Dissemination		
Infected, untreated (males)	Same as in Table 3	Mean	20.3
Infected, treated with 0.1 ml aqueous vehicle of cortisone acetate every 48 hrs starting 2 days after infection (males)	28	Mean	30.4
	28	Av. dev. from mean	0.96
	31	Stand. dev. from mean	1.2
	31		
	31	*t* for infected, treated males compared to infected, untreated males	—2.35
	31		
	31		
	31		
	31	Degrees of freedom	18
	31	P	.05—.02
Uninfected, treated as above (males)	Same as in Table 2	Mean	0.0
Infected, untreated (females)	Same as in Table 3	Mean	19.5
Infected, treated with 0.1 ml aqueous vehicle of cortisone acetate every 48 hrs starting 2 days after infection (females)	3	Mean	17.7
	3	Av. dev. from mean	11.7
	5	Stand. dev. from mean	12.0
	6		
	13	*t* for infected, treated females compared to infected untreated females	0.34
	27		
	30		
	30	Degrees of freedom	18
	30	P	.80—.70
	30		
Uninfected, treated as above (females)	Same as in Table 2	Mean	0.0

one is the actual decrease of the severity of the infection; the other is the result of its toxicity at the levels used, a toxicity manifest either by aggravating an existing infection or by exciting a dormant or latent infection. If the mean dissemination value for infected but untreated mice is lower than that which results form the toxic effects of the cortisone, then the efficacious effect of cortisone is masked. We know that the aqueous vehicle enhances the severity of the infection, but since, in this work, this enhancement was not greater than the level of toxicity due to cortisone (in mice in which only a mild to moderate monilial infection had been induced), again any efficacious effect due to cortisone could not be detected by comparing the data for infected mice treated with cortisone and those treated with aqueous vehicle.

If the above postulates have any validity, it should be possible to decrease the severity of an infection in a group of mice which has a mean dissemination value greater than that due to the toxicity of cortisone. Also, regardless of the severity of infection, the use of aqueous vehicle alone should still enhance it. This is exactly what seems to have occurred when a severe monilial infection was induced in mice with the aid of a mucin adjuvant. Thus, a mean dissemination value due to moniliasis of 20.5 for male mice was reduced to 11.6 by the same dosage schedule of cortisone as used for the mice mildly infected, and 11.6 again is equivalent to that value which represents the toxic level for cortisone on normal mice (12.3). The use of aqueous vehicle alone raised the mean value for male mice from 20.5 to 30.4 (table 4).

The role that the aqueous vehicle plays in enhancing the infection is not clear. A possibility not yet excluded is that the toxic effects of cortisone may in part be attributed to some component or components of the aqueous vehicle. KILBOURNE (1951a) found no increase in virus concentration in embryonated eggs injected with the aqueous vehicle of cortisone containing 1.5 % benzyl alcohol. HOSINO (1941) reported that benzyl alcohol, injected into rats daily for 40 days, caused a gradual decrease in blood sugar. It is not known as yet whether a hypolgycemia occurred in the mice under the conditions of the experiments reported here or whether, having been induced, this would be determinetal to a host with monilial involvement. These factors might profitably be investigated for their possible significance in clinical therapy.

Sex differences are also present in the various portions of this work. Male animals, both mildly and severely infected, showed a marked increase in severity of infection when treated with aqueous vehicle (compare tables 2 and 4). Female mice under the same conditions showed a very small or no enhancement of the mean dissemination values; thus they appear to be more resistant to the deleterious synergistic activity between the aqueous vehicle and monilial involvement.

Cortisone treatment markedly reduced the mean dissemination value of severely infected male mice, but left the severity of infection for similarly infected female mice relatively unchanged. This might indicate that it would take more cortisone to reduce an infection in female mice than to effect a comparable reduction in male animals. This might in some away be due to the higher resistance shown by female animals to the toxic effects of cortisone. This higher resistance has already been demonstrated by SCHERR (1952) as occurring at moderate and increased environmental temperatures.

The temperature of the animal quarters during the time such experiments are performed plays a significant role in the results. An increased temperature (37° C) or cold temperature (5° C) enhances the mortality of *Candida*-infected mice as compared to that found at intermediate temperatures (20°, 25° C)

(SCHERR, 1953b). The effect of temperature on the toxicity of cortisone has already been mentioned. It is thus important to consider the general temperature range at the time the experiments reported here were run.

The peak temperatures for any day, obtained from U.S. Department of Commerce weather charts[1]), have been plotted against the total number

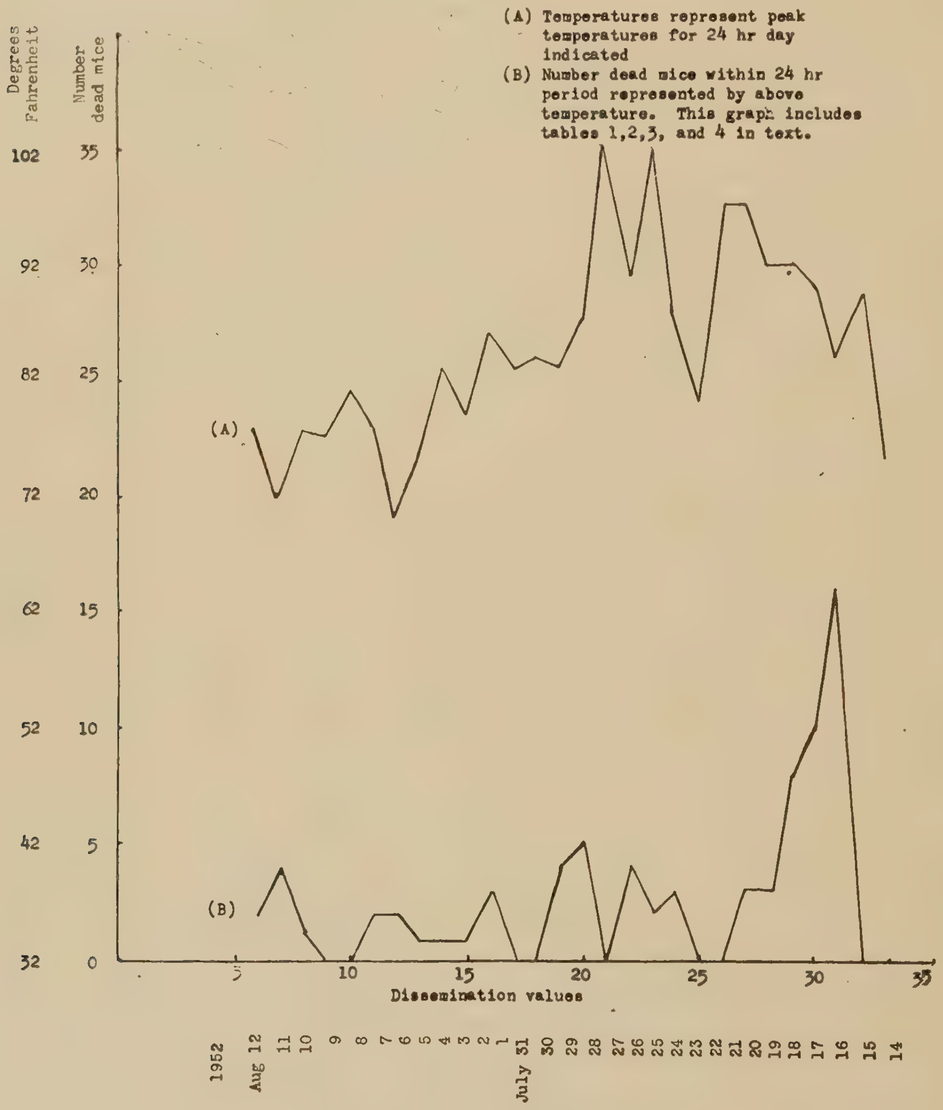

Fig. 1. Correlation Between Environmental Temperature and Mortality of *Candida*-Infected Mice and Controls.

of dead experimental mice found on that day (figure 1). In addition, dissemination values are indicated on the ordinate recording the dates during which the experiments were in progress so that, by sight examination of any of the tables, one may determine the day an animal died and the peak temperature for that day for any given dissemination value.

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[1]) The city temperatures are similar to the temperatures in the animal rooms for those days warm enough so as not to require heating.

Despite the major influences of monilial infection, toxicity of the cortisone, and the enhancing effect of the aqueous vehicle, factors which because of their severity might in a number of instances overcome temperature differences, there are numerous points of correlation (figure 1) between the crests and troughs on the temperature curve and those on the mouse mortality curve. Other such figures have been drawn for similar experiments done during the summer months with similar correlation between sudden peaks of hot weather and mortality of mice.

The work reported here lends itself to a hypothesis that cortisone may affect the course of certain infectious diseases in at least two ways. On the one hand the severity of the infection may be enhanced, and on the other it may be diminished. These contesting factors result in an infection whose final course is dependent upon those conditions and environment of the host which favor one or the other. In line with our observations it is significant to note that DUFFY and MORGAN (1951) reported that rabbits injected with *Shiga dysenteriae* endotoxin showed a reduced febrile response or an aggravated febrile and systemic response to cortisone, depending upon the time-dose relationship between the toxin and the cortisone. Also, the feeling of "well-being" reported by numerous investigators for their patients receiving cortisone (or ACTH) therapy probably reflects some efficacious effect on the infectious process despite the fact that the disease persists, often in a more severe form.

Since a number of methods have already been reported for antagonizing the deleterious effect of cortisone on normal animals, e.g., a cold environment (SCHERR, 1952), somatrophic hormone (SELYE, 1951), whole liver (ERSHOFF, 1951), it should be profitable to determine whether the use of any of these procedures will permit an efficacious effect of cortisone on an infected animal by some preferential antagonism of the deleterious property of the cortisone. This approach is being studied at present and will be reported in a subsequent paper.

Summary

A mild to moderate moniliasis in mice was rendered more severe by periodic treatment with cortisone acetate. This enhanced severity of infection occurring in male and female animals was no greater, however, than could be atributed to the deleterious effect of the cortisone *per se*, as evidenced by the results on uninfected animals treated similary with cortisone. Infected male animals treated periodically with the aqueous vehicle of cortisone revealed an enhanced severity of monilial involvement; female mice similarly infected and treated showed no modification of the severity of infection. A severe moniliasis induced by suspending the *Candida* cells in mucin was reduced in severity for male, but not for female mice by the same schedule of cortisone treatment as used above. The effect of the aqueous vehicle on such a severe monilial involvement was similar to that found with mildly infected mice. The possible significance of these observations are discussed.

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The Effect of Cortisone on the Course of Systemic Moniliasis in Mice[1]

II. An Attempt to Reverse the Toxic Effect of Cortisone with Lowered Environmental Temperature or Somatotrophic Hormone (STH)

by

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In a previous paper Scherr (1953b) reported that cortisone acetate reduces the severity of acute disseminated moniliasis in male mice, but not in female mice. Treatment with cortisone of mice which were mildly infected with *Candida albicans* 171D resulted in an enhanced dissemination of the disease in both sexes. The hypothesis was proposed by Scherr that cortisone may elicit either a deleterious or efficacious effect on moniliasis, depending upon the conditions and environment of the host. In accord with these findings Duffy and Morgan (1951) reported that rabbits injected with *Shiga dysenteriae* endotoxin showed a reduced febrile response or an aggravated febrile and systemic response to cortisone, depending upon the time-dose relationship between the toxin and the cortisone.

A reversal of the toxic effects of cortisone has been accomplished with cold environmental temperature (Scherr, 1952), somatotrophic hormone (Selye, 1951a), and whole liver (Ershoff, 1951). The work reported here represents an attempt to antagonize the deleterious effect of cortisone on *Candida*-infected mice withour impairing its efficacious effect, presuming that such a distinction for cortisone may properly be made, by changes in environmental temperature and the use of STH.

Materials and Methods

The materials and methods used were essentially those previously described by Scherr (1952, 1953b). All animals were treated intramuscularly with the preparations used. In all cases treatment was instituted the same day the mice were infected. Cortisone (Cortone (R) acetate, Merck) was prepared in an aqueous vehicle containing 0.5 % benzyl alcohol and 5 mg cortisone acetate per ml of solution; the concentration of the other ingredients of Aqueous Vehicle No. 1 (Merck) remained the same [2]. Animals treated with cortisone suspended in the aqueous vehicle received 0.1 ml (0.5 mg cortisone) every 48 hours. Control animals treated only with the aqueous vehicle containing 0.5 % benzyl alcohol received 0.08 ml every 24 hours. Animals treated solely with STH ("Somatotrofin", Frank W. Horner, Ltd, Montreal) were inoculated daily with 0.05

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[2] Aqueous Vehicle No. 1 (MERCK), the vehicle in which cortisone acetate was originally suspended, contained 1.5 % benzyl alcohol. A note from Dr. Elmer Alpert, Manager of Clinical Research of Merck and Co., Inc., informs me that the present vehicle for Cortisone (R) acetate, likewise designated Aqueous Vehicle No. 1, contains 0.9 % benzyl alcohol.

ml (0.1 mg) of a solution of STH in the aqueous vehicle containing 0.5 % benzyl alcohol. In order to avoid the necessity of 2 separate inoculation into mice treated with cortisone ans STH, these animals were inoculated with a mixture of cortisone and STH on one day and with STH the following day; this alternating schedule met the equivalent dosage requirements given to animals being treated only with cortisone or STH. The only discrepancy in dosage to be found among these groups of treated animals concerns the quantity of aqueous vehicle each animal received. Thus, the volume in ml of aqueous vehicle inoculated every 48 hours into mice treated with the aqueous vehicle, cortisone, STH, and cortisone and STH was 0.16, 0.1, 0.1, and 0.15, respectively. No uninfected mice were treated with aqueous vehicle because it has already been shown (Scherr, 1953b) that 0.1 ml of aqueous vehicle containing 1.5 % benzyl alcohol inoculated every 48 hrs. is not deleterious to normal mice, as far as could be determined by mortality data and macroscopic examination at autopsy. No uninfected mice were treated with cortisone, cortisone and STH, or STH, chiefly because this study, due to the large number of variables requiring control, could not hope to be anything more than exploratory at this stage; because there is sufficient previous data to permit an accurate estimate of the deleterious effect of cortisone on normal mice (Scherr; 1952, 1953b), and, not of least importance, because the amount of STH at our disposal at the time this study was done was limited.

Results

The effect of environmental temperature.

Tables 1 and 2 record the results of the experiment in which male and female mice, infected with both a mild and a moderate monilial infection, were treated with cortisone. Half of the animals were kept at a temperature of 20°—25°C and half at 0°—5°C. In each case 10 mice comprised and experimental group.

The mild infections in both male and female mice kept at 20°—25°C environmental temperature were enhanced by treatment with cortisone; in the females this was possibly not to the extent found previously (Scherr, 1953a) or reported subsequently in this report (table 3). An infection of moderate severity in both sexes was not significantly modified in intensity by treatment with cortisone. In the data previously reported (Scherr, 1953a) and also in subsequent data presented in this report (table 4) it is shown that a very severe infection is reduced in intensity be treatment with cortisone. Thus, cortisone enhances the severity of a mild monilial infection, does not alter the severity of a moderate infection, and is definitely efficacious to a certain extent in a severe infection.

All animals kept at 0°—5°C died within 2 days after the experiment was instituted. Animals infected with moniliasis and kept in a cold environment suffer a more severe dissemination than those kept at room temperature (Scherr, 1953a). However, normal animals which would die in 1 or 2 days at environmental temperatures similar to those used here would be expected to survive if treated with cortisone (Scherr, 1952).

Table 1. *Effect of Environmental Temperature on Mice Infected with Candida albicans and Treated with Cortisone* (*Duration of experiment* — 20 *days*).

Legend	Dissemination		
Infected,	0	Mean	2.5
untreated.	1	Av. dev. from mean	2.2

Table 1 (continued).

Legend	Dissemination		
Kept at	1	Stand. dev. from mean	3.6
20°—25°C	1		
(males)	1		
	1		
	2		
	2		
	3		
	13		
Infected,	1	Mean	6.3
treated with	2	Av. dev. from mean	3.9
0.50 mg	2	Stand. dev. from mean	4.3
cortisone	2		
every 48 hrs.	5	*t* for infected, untreated males	
Kept at	7	compared to infected treated	
20°—25°C	9	males	—2.04
(males)	11	Degrees of freedom	18
	11	P	.10—.05
	13		
Infected,	23	Mean	23.0
untreated.	23	Av. dev. from mean	0.0
Kept at	23	Stand. dev. from mean	0.0
0°—5°C	23		
(males)	23		
	23		
	23		
	23		
	23		
	23		
Infected,	23	Mean	23.0
treated with	23	Av. dev. from mean	0.0
0.5 mg	23	Stand. dev. from mean	0.0
cortisone	23		
every 48 hrs.	23		
Kept at	23		
0°—5°C	23		
(males)	23		
	23		
	23		
Infected,	0	Mean	2.8
untreated.	0	Av. dev. from mean	2.9
Kept at	1	Stand. dev. from mean	4.5
20°—25°C	1		
(females)	1		
	1		
	2		
	2		
	4		
	16		
Infected,	0	Mean	4.8

Table 1 (continued).

Legend	Dissemination		
treated with	0	Av. dev. from mean	4.2
0.50 mg	1	Stand. dev. from mean	4.6
cortisone	1		
every 48 hrs.	3	*t* for infected, untreated females compared to infected, treated females	—.093
Kept at	3		
20°—25°C	7		
(females)	9	Degrees of freedom	18
	11	P	.50—.30
	13		
Infected,	23	Mean	23.0
untreated	23	Av. dev. from mean	0.0
Kept at	23	Stand. dev. from mean	0.0
0°—5°C	23		
(females)	23		
	23		
	23		
	23		
	23		
	23		
Infected,	24	Mean	24.0
treated with	24	Av. dev. from mean	0.0
0.50 mg	24	Stand. dev. from mean	0.0
cortisone	24		
every 48 hrs.	24		
Kept at	24		
0°—5°C	24		
(females)	24		
	24		
	24		
Uninfected,	23	Mean	23.0
untreated.	23	Av. dev. from mean	0.0
Kept at	23	Stand. dev. from mean	0.0
0°—5°C	23		
(males)	23		
	23		
	23		
	23		
	23		
	23		
Uninfected,	23	Mean	23.0
untreated.	23	Av. dev. from mean	0.0
Kept at	23	Stand. dev. from mean	0.0
0°—5°C	23		
(females)	23		
	23		
	23		
	23		
	23		
	23		

TABLE 2. *Effect of Environmental Temperature on Mice Infected with Candida albicans Suspended in Mucin and Treated with Cortisone (Duration of experiment — 20 days)*

Legend	Dissemination		
Infected, untreated. Kept at 20°—25°C (males)	1	Mean	9.4
	2	Av. dev. from mean	7.9
	3	Stand. dev. from mean	8.8
	3		
	4		
	4		
	10		
	21		
	23		
	23		
Infected, treated with 0.50 mg cortisone every 48 hrs. Kept at 20°—25°C (males)	0	Mean	10.4
	7	Av. dev. from mean	3.2
	9	Stand. dev. from mean	4.2
	10		
	10		
	12		
	13		
	13		
	15		
	15		
Infected, untreated. Kept at 0°—5°C (males)	24	Mean	24.0
	24	Av. dev. from mean	0.0
	24	Stand. dev. from mean	0.0
	24		
	24		
	24		
	24		
	24		
	24		
	24		
Infected, treated with 0.50 mg cortisone every 48 hrs. Kept at 0°—5°C (males)	23	Mean	23.0
	23	Av. dev. from mean	0.0
	23	Stand. dev. from mean	0.0
	23		
	23		
	23		
	23		
	23		
	23		
	23		
Infected, untreated. Kept at 20°—25°C (females)	1	Mean	9.6
	3	Av. dev. from mean	8.0
	3	Stand. dev. from mean	9.0
	3		
	4		
	4		
	9		

Table 2 (continued).

Legend	Dissemination		
	23		
	23		
	23		
Infected, treated with 0.50 mg cortisone every 48 hrs. Kept at 20°—25°C (females)	5	Mean	13.8
	7	Av. dev. from mean	5.4
	9	Stand. dev. from mean	6.3
	11		
	11	t for infected, untreated females compared to infected, treated females kept at 20°—25°C	—1.15
	13		
	15		
	19	Degrees of freedom	18
	24	P	.30—.20
	24		
Infected, untreated. Kept at 0°—5°C (females)	24	Mean	24.0
	24	Av. dev. from mean	0.0
	24	Stand. dev. from mean	0.0
	24		
	24		
	24		
	24		
	24		
	24		
	24		
Infected, treated with 0.50 mg cortisone every 48 hrs. Kept at 0°—5°C (females)	24	Mean	24.0
	24	Av. dev. from mean	0.0
	24	Stand. dev. from mean	0.0
	24		
	24		
	24		
	24		
	24		
	24		
	24		

It is noteworthy that the added stress of monilial infection, even in those animals lightly infected, was sufficient to overcome the beneficial effect of cortisone for animals kept at freezing temperatures. Whether use of higher doses of cortisone would change this picture cannot be stated at this time, but this might be anticipated since if:

1) Cortisone reverses the deleterious effect of cold on normal mice.

2) A low environmental temperature enhances the severity of a mild monilial infection.

3) Cortisone is beneficial to mice severely infected with moniliasis.

Thus it might be expected that an optimum concentration of cortisone can be found which would reverse the deleterious effects of cold environment on *Candida*-infected mice.

The Effects of STH

The data for the work attempting to antagonize the deleterious effect of cortisone with STH are recorded in tables 3 and 4 and graphically illustrated in figures 1, 2, 3, and 4. In this work 20 mice comprised an experimental group and all animals were kept at room temperature (23°C±5°C).

For male mice mildly infected the effect of cortisone was to enhance the severity of the infection (compare groups 4—34 A and 4—34 D in table 3; note figure 1). This is in accord with previous findings (Scherr, 1953b). This increase in severity must be attributed solely to the cortisone, since the

FIG. I

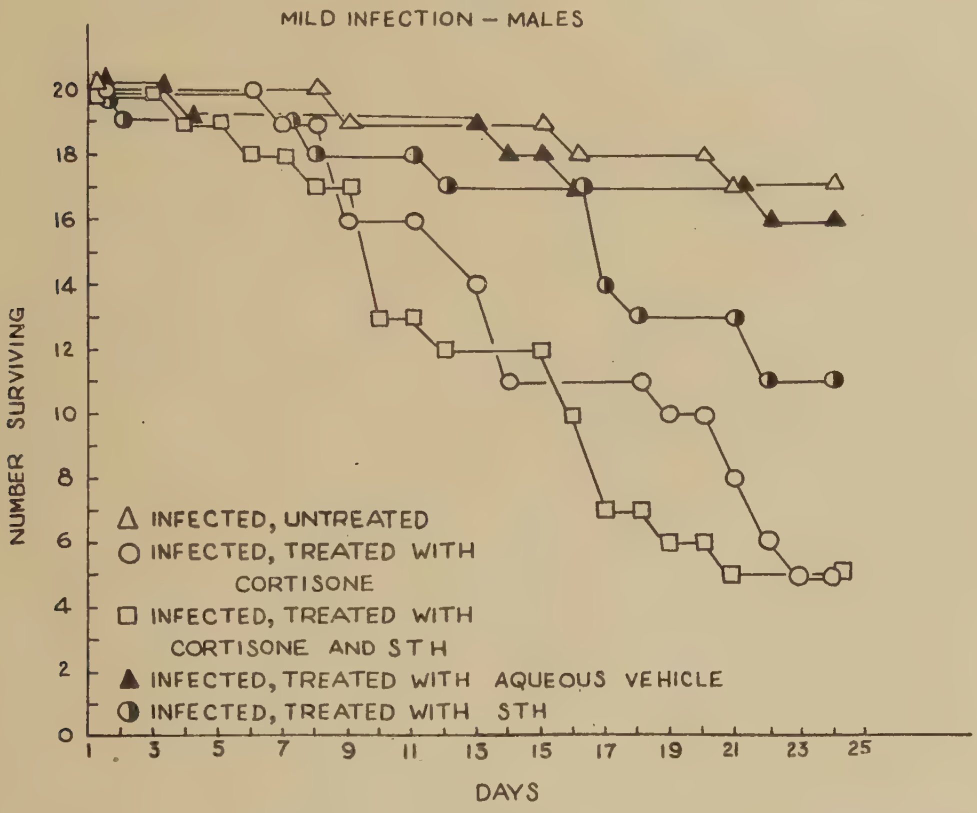

Table 3. *The Effect of Somatotrophic Hormone (STH) on Mice Infected with Candida albicans and Treated with Cortisone (Duration of experiment — 24 days)*

Legend[1] and code number for group	Dissemination			
Infected,	0	2	Mean	3.7
untreated	0	2	Av. dev. from mean	3.4
(males)	1	3	Stand., dev. from mean	5.1
4—34 A	1	3		
	1	3		
	1	3		
	1	4		

Table 3 (continued).

Legend and code number for group	Dissemination			
	1	9		
	1	14		
	2	21		
Infected, treated with cortisone (males) 4—34 D	—	15	Mean	12.9
	—	16	Av. dev. from mean	6.1
	2	16	Stand. dev. from mean	6.8
	2	16		
	3	17	*t* for 4—34A compared to 4—34D	—4.62
	7	18	Degrees of freedom	36
	8	21	P	∠.001
	8	21		
	9	21		
	9	23		
Infected, treated with cortisone and STH (males) 4—38 C	0	14	Mean	13.7
	3	14	Av. dev. from mean	6.2
	4	18	Stand. dev. from mean	7.4
	4	20		
	5	20		
	9	20		
	11	20		
	13	22		
	13	24		
	13	26		
Infected, treated with STH (males) 4—59 A	0	4	Mean	7.8
	0	8	Av. dev. from mean	6.5
	0	8	Stand. dev. from mean	7.8
	0	12		
	1	13	*t* for 4—34A compared to 4—59A	—1.9
	3	13	Degrees of freedom	38
	3	13	P	0.10—.05
	3	18		
	3	22		
	4	28		
Infected, treated with aqueous vehicle (males) 4—34 F	—	2	Mean	5.0
	—	3	Av. dev. from mean	4.9
	0	3	Stand. dev. from mean	6.7
	0	3		
	1	3	*t* for 4—34A compared to 4—34F	—0.66
	1	3	Degrees of freedom	36
	1	8	P	.70—.50
	2	14		
	2	16		
	2	26		
Infected, untreated (females) 4—34 O	0	3	Mean	4.9
	0	4	Av. dev. from mean	4.2
	1	4	Stand. dev. from mean	6.3
	1	4		
	1	5		

Table 3 (continued)

Legend and code number for group	Dissemination			
	1	5		
	2	5		
	2	12		
	2	20		
	2	24		
Infected, treated with cortisone (females) 4—34 R	—	13	Mean	12.7
	—	13	Av. dev. from mean	4.4
	3	13	Stand. dev. from mean	6.0
	3	13		
	4	13	*t* for 4—34O compared to 4—34R	—.3.8
	8	16	Degrees of freedom	36
	9	16	P	∠.001
	11	20		
	12	24		
	12	25		
Infected, treated with cortisone and STH (females) 4—38 G	—	9	Mean	9.0
	2	9	Av. dev. from mean	5.5
	2	10	Stand. dev. from mean	6.6
	3	12		
	3	12	*t* for 4—34R compared to 4—38G	1.74
	3	13	Degrees of freedom	35
	4	15	P	.10—.05
	4	17		
	4	19		
	4	26		
Infected, treated with STH (females) 4—59 C	0	2	Mean	1.8
	0	3	Av. dev. from mean	1.5
	0	3	Stand. dev. from mean	1.6
	0	3		
	0	3	*t* for 4—34 O compared to 4—59C	1.72
	0	3	Degrees of freedom	38
	0	3	P	.10—.05
	1	4		
	1	4		
	1	4		
Infected, treated with aqueous vehicle (females) 4—34 T	1	5	Mean	8.9
	2	5	Av. dev. from mean	6.8
	3	5	Stand. dev. from mean	8.0
	3	5		
	3	11		
	3	16		
	3	20		
	2	20		
	3	24		
	5	27		

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¹) For details of dosages and schedules see text.

TABLE 4. *The Effect of Somatotrophic Hormone (STH) on Mice Infected with Candida albicans Suspended in Mucin and Treated with Cortisone (Duration of experiment — 24 days)*

Legend[1] and code number for group	Dissemination			
Infected, untreated (males) 4—34 B	3	26	Mean	23.3
	4	26	Av. dev. from mean	5.3
	12	26	Stand. dev. from mean	7.5
	21	28		
	24	28		
	24	28		
	25	28		
	26	28		
	26	28		
	26	28		
Infected, treated with cortisone (males) 4—34 H	4	18	Mean	17.9
	12	20	Av. dev. from mean	5.1
	12	20	Stand. dev. from mean	6.2
	12	20		
	12	22	*t* for 4—34B compared to 4—34H	2.4
	12	23	Degrees of freedom	38
	13	24	P	.02—.01
	16	27		
	17	28		
	18	28		
Infected, treated with cortisone and STH (males) 4—38 D	2	26	Mean	23.7
	9	26	Av. dev. from mean	4.6
	18	27	Stand. dev. from mean	6.6
	20	27		
	23	27		
	26	27		
	26	27		
	26	28		
	26	28		
	26	28		
Infected, treated with STH (males) 4—59 B	0	24	Mean	19.5
	4	27	Av. dev. from mean	7.7
	4	27	Stand. dev. from mean	9.9
	8	27		
	8	27		
	17	28		
	17	28		
	17	28		
	19	28		
	24	28		
Infected, treated with aqueous vehicle (males) 4—34 J	4	22	Mean	19.6
	5	24	Av. dev. from mean	7.1
	9	26	Stand. dev. from mean	8.2
	9	27		
	11	28		
	15	28		

Table 4 (continued).

Legend and code number for group	Dissemination			
	16	28		
	18	28		
	18	28		
	20	28		
Infected, untreated (females) 4—34 P	—	24	Mean	18.6
	2	24	Av. dev. from mean	9.3
	3	26	Stand. dev. from mean	10.5
	4	26		
	4	26		
	5	26		
	5	28		
	20	28		
	22	28		
	24	28		
Infected, treated with cortisone (females) 4—34 V	—	20	Mean	20.3
	9	23	Av. dev. from mean	5.4
	12	23	Stand. dev. from mean	5.7
	12	23		
	15	25		
	15	28		
	16	28		
	16	28		
	17	28		
	19	28		
Infected, treated with cortisone and STH (females) 4—38 H	0	14	Mean	14.7
	1	17	Av. dev. from mean	8.4
	5	17	Stand. dev. from mean	9.6
	5	22		
	5	22	*t* for 4—34V compared to 4—38H	2.15
	5	27	Degrees of freedom	37
	7	27	P	.05—.02
	7	28		
	14	28		
	14	28		
Infected, treated with STH (females) 4—59 D	0	26	Mean	20.3
	4	27	Av. dev. from mean	7.2
	8	27	Stand. dev. from mean	8.4
	16	27		
	17	27		
	17	28		
	17	28		
	17	28		
	18	28		
	24	28		
Infected, treated with aqueous	2	24	Mean	19.1
	5	27	Av. dev. from mean	8.7
	5	28	Stand. dev. from mean	9.8

Table 4 (continued).

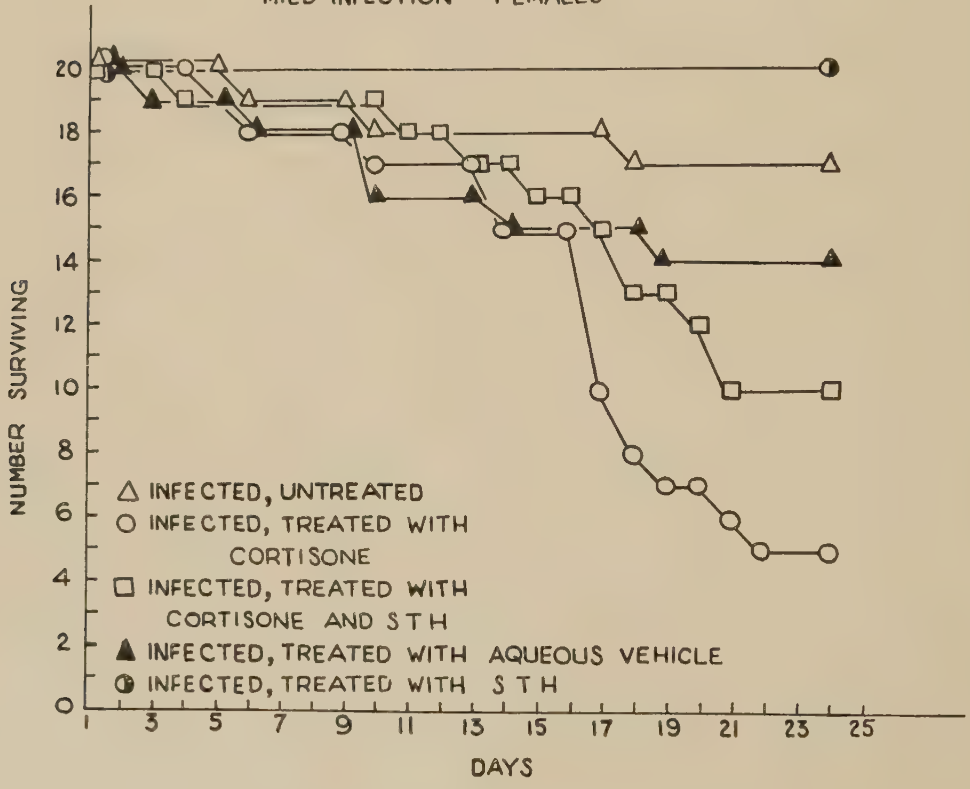
Legend and code number for group	Dissemination	
vehicle	5	28
(females)	5	28
4—34 X	10	28
	17	28
	18	28
	18	28
	22	28

[1]) For details of dosages and schedules see text.

aqueous vehicle, at the lowered concentrations of benzyl alcohol used here, did not modify the severity of the disease. The treatment of infected males with cortisone and STH did not antagonize the deleterious effect of cortisone as was expected (compare groups 4—34 D and 4—38 C in table 3). This might be due to the fact that STH will enhance the severity of infection to a certain degree, as evidenced by the data of group 4—59 A in table 3, or might be due to some condition of the host resulting from such multiple interrelated factors as sex, environmental temperature, severity of infection, and cortisone dosage and schedule of inoculation.

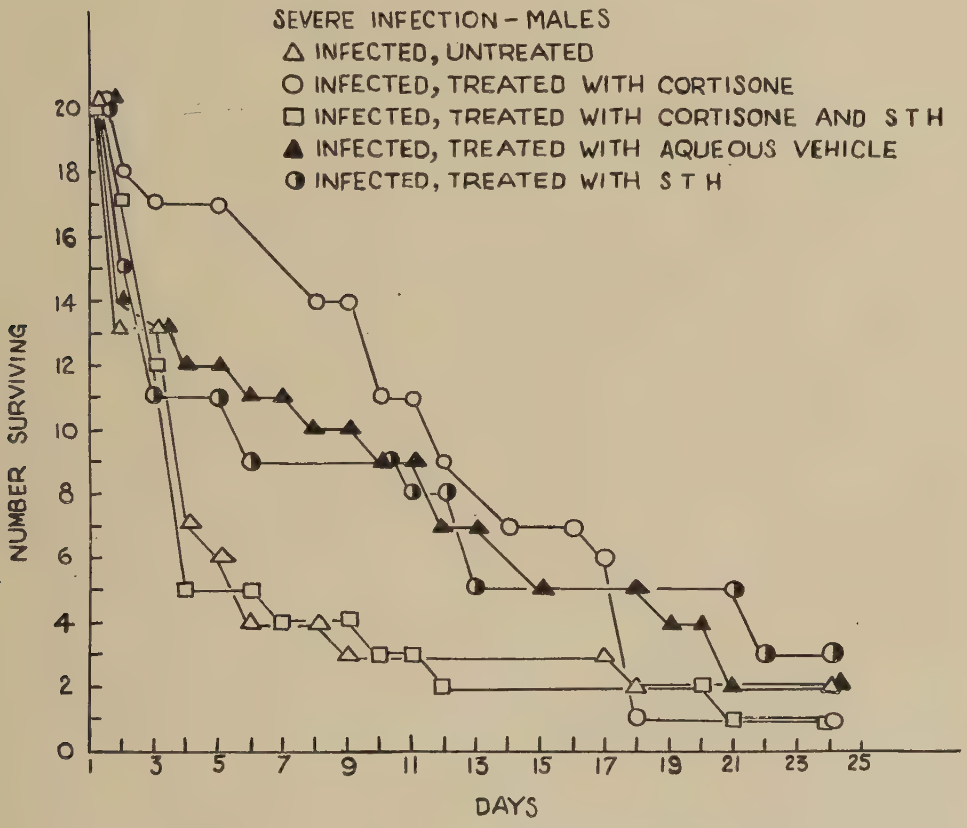
FIG. 2

MILD INFECTION – FEMALES



Female mice mildly infected and treated similarly to the male animals also showed an enhanced severity of infection upon treatment with cortisone (compare group 4—30 O and 4—34 R in table 3; note figure 2). This finding again is in accord with previous data (SCHERR, 1953b). The effect of STH on infected female mice treated with cortisone was to reduce, to a certain extent, the deleterious effect of the cortisone (compare groups 4—34 R and 4—38 G in table 3; note figure 2). In contrast to that found for male animals, infected female mice treated solely with STH did not show any enhanced severity of infection; rather they appeared to be protected to a significant degree by this regimen of STH inoculations. There was an increase in the severity of infection in the females treated solely with the aqueous vehicle, but not to a significant degree.

FIG. 3



If a severe infection is induced and the same treatment schedules as described above are followed, the results are quite different from those found in mildly infected animals.

Male mice thus infected and treated with cortisone show a reduced severity of infection (compare groups 4—38 B with 4—34 H in table 4; note figure 3). This observation is also in accord with our previous data (SCHERR, 1953b). The effect of STH, used concomitantly with cortisone, on severely infected male mice was to reverse completely the efficacious effect of cortisone. This is indicated in table 4 but is most vividly demonstrated in figure 3. The use of STH alone resulted in a decreased severity of infection and mortality rate, approximately one-half as effective as cortisone alone (compare groups 4—34 B and 4—59 B in table 4). However, it is not possible to attribute this reduction solely to the effect of STH, since the aqueous vehicle, when used alone, was able to reduce the severity of infection in these male animals to the same degree as that found with STH (compare tables 4—34 B, 4—59 B, and 4—34 J in table 4; note figure 3).

Treatment with cortisone of female mice in which a severe infection had been induced resulted in a dissemination value which indicates that there was a very slight increase in severity of infection (compare groups 4—34 P and 4—34 V in table 4). Figure 4 indicates that this similarity in dissemination values between infected, untreated female mice and infected mice treated with cortisone results from a cancelling out of a moderate decrease in mortality rate during the first half of the experimental period followed by an increased mortality rate during the latter half of the experimental period. The role of STH, when inoculated into infected female mice being treated with cortisone, was apparently to antagonize in part the deleterious effects of cortisone without impairing the efficacious effect of cortisone, since the survival curve for female mice infected with moniliasis and treated with cortisone and STH is higher than that for either infected, untreated mice or infected mice treated with cortisone (figure 4). In addition, this enhanced survival rate could not be attributed to an efficacious effect of STH since, when used alone, it did not enhance the survival rate of infected mice but actually was deleterious to a certain extent (compare groups 4—34 P and 4—59 D in table 4; note figure 4). Aqueous vehicle did not significantly modify the course of infection (group 4—34 X in table 4).

Discussion

The failure to preferentially antagonize the deleterious effects of cortisone by keeping mice infected with moniliasis at lowered environmental temperatures does not negate the potentiality of this approach for other infectious diseases since the severity of moniliasis is markedly affected by a cold environmental temperature. It might be more profitable to explore this temperature effect with infectious diseases whose severity is not greatly altered by a cold environmental temperature. Such studies are in progress at the present time.

It is pertinent to note here that the effect of changes in environmental temperature on an infected animal is complicated by a number of factors. Thus Ipsen (1951, 1952) has shown that although a warm environmental temperature enhanced the toxic effects of tetanus toxin for mice, it also increased the production of antibodies. A low temperature delayed the effect of intoxication but the animals ultimately died, with lower doses of toxin than would be lethal at higher temperatures.

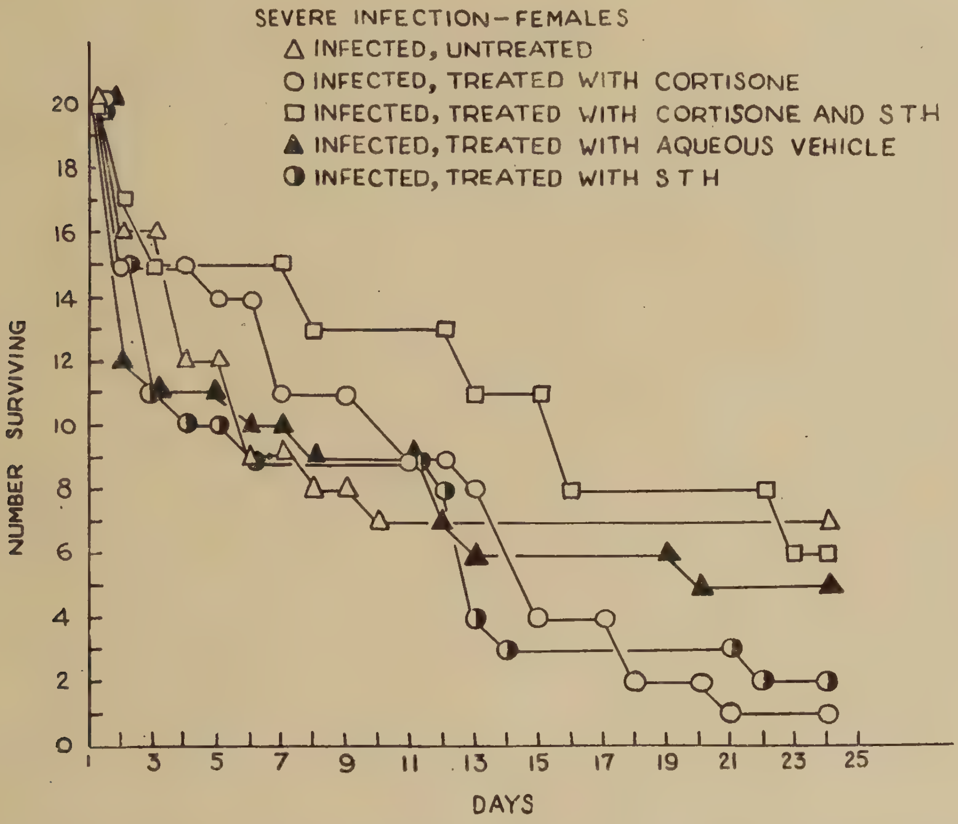
Mills and Schmidt (1942) reported that mice kept at 65°F showed a higher incidence of survival after infection with Type I pneumococcus than animals kept at 75°F or 90°F. This difference in survival could not be attributed to the effect of changes in body temperature on the growth of the pneumococcus, since the mean rectal temperatures for mice in the hot and cold rooms were essentially the same.

The data presented here is complicated by the fact that we are dealing with multiple factors, all of which are probably interrelated. We already know that the following factors must be controlled:

1) Severity of infection.
2) Sex of animal.
3) Amount of cortisone and its dosage schedule.
4) Amount of STH and its dosage schedule.
5) Type of aqueous vehicle.
6) Environmental temperature.

Our data also suggests the well known fact that animals treated with hormones periodically and over an extended period of time may become tolerant to these agents. Note that the curve for mice treated with cortisone (figure 3) falls stepwise, whereas the infected and untreated animals show a precipitous fall in survivors within the first few days, followed by a general leveling off.

FIG. 4



A similar picture is presented in figure 4 for infected mice treated with cortisone and STH. Whether periodic increments in cortisone and/or STH will modify the mortality curves presented here needs to be determined.

The fact that STH will antagonize the effect of cortisone is most vividly illustrated here for severely infected male mice (figure 3). Only in severely infected female mice treated with cortisone and STH was it possible to realize, albeit to a moderate degree, our premise that it might be possible to preferentially antagonize the deleterious effects of cortisone on mice infected with moniliasis by using STH. The reason this same phenomenon was not observed with male animals similarly infected and treated is obscure, but may be due to the fact that male mice are more susceptible to the deleterious effects of cortisone (SCHERR, 1952), and thus require a greater quantity of STH than used here to reverse its effect.

Since the sex of the animal is a significant factor in these experiments it might be appropriate to modify the "maleness" or "femaleness" of the experimental animals by pre-treatment with certain hormones. Studies using gonadotropic hormones which have been conducted by this author for some time, indicate that under certain conditions they will modify the course of systemic moniliasis in mice in a favorable manner and that this effect is also influenced by the sex of the animal (SCHERR and WEAVER, 1951; SCHERR, unpublished data). TALALAY *et al.* (1952) have shown that cortisone inoculated into dogs together with diethylstilbesterol will suppress, to a certain extent, the stimulating effect of the estrogen on the uterus. TONUTTI (1950) reported that diphtheria toxin inoculated into immature guinea pigs will not induce any marked changes in the testes unless the animals are pretreated with gonadotropins, then a hemorrhagic necrosis occurs. Whether such observations might represent significant factors for the modification of the pathogenesis of certain diseases by cortisone is not clear at this time.

The marked difference in mortality rates between groups of mice infected with *C. albicans* suspended in saline and those infected with the organisms in a 5 % mucin vehicle is, to a certain extent, in accord with the observations of SALVIN (1952). SALVIN, however, used killed cells of *C. albicans* and concluded that death was due to an endotoxin. Yet it would appear that this endotoxin required an adjuvant of one kind or another in order for death of the animals to occur. Such an intoxication might be expected to take place under the conditions of the experiments reported here and, in the light of work such as that of DUFFY and MORGAN (1951), one might expect it to be influenced by cortisone treatment. It is not unreasonable to submit an additional possibility for SALVIN's results. Since the mucin adjuvant and killed cells of *C. albicans* result in a synergistic effect, there is as much justification in concluding that the killed cells were acting as adjuvant to the mucin (or other adjuvants used) as the converse.

Summary

1. It was not possible to protect mice from the lethal effects of a cold environment by using cortisone if the mice had previously been infected with moniliasis. This was true whether the induced infection was a mild or severe one.

2. Only in female mice infected with moniliasis and treated with cortisone and STH was it possible to demonstrate a reversal of the deleterious effect of cortisone on *Candida*-infected mice. In female mice severely infected and treated with cortisone and STH, the mortality rate was less severe than that found for female mice infected with moniliasis and treated with cortisone or female mice infected but untreated.

3. Male mice severely infected profited from treatment with cortisone by showing a reduced mortality rate but this effect was completely reversed by treating infected male mice with cortisone and STH.

4. The significance and possible role of the interrelated factors of severity of infection, sex of animal, amount of cortisone and its dosage schedule, amount of STH and its dosage schedule, type of aqueous vehicle, and environmental temperature are discussed.

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